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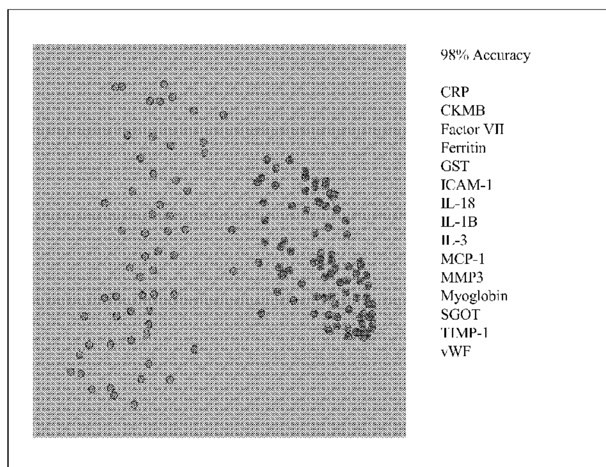
(54) Title: METHODS AND KITS FOR THE DIAGNOSIS OF STROKE



(57) Abstract: Provided are methods for the detection and diagnosis of stroke. The methods are based on the discovery that abnormal levels of selected analytes in sample fluid, typically blood samples, of patients who are at risk are supportive of a diagnosis of stroke. At least two new biomarkers for stroke are thus disclosed, MMP-3 and SGOT. Altogether the concentrations of twelve analytes provide a sensitive and selective picture of the patient's condition, namely, whether the patient is suffering a stroke. Other important biomarkers for stroke are described, including but not limited to IL-18, Factor VII, ICAM-I, Creatine Kinase-MB, MCP-I, Myoglobin, C Reactive Protein, von Willebrand Factor, TIMP-I, Ferritin, Glutathione S-Transferase, Prostate Specific Antigen (free), IL-3, Tissue Factor, alpha-Fetoprotein, Prostatic Acid Phosphatase, Stem Cell Factor, MIP-I -beta, Carcinoembryonic Antigen, IL-13, TNF- alpha, IgE, Fatty Acid Binding Protein, ENA-78, IL-I -beta, Brain-Derived Nerotrophic Factor, Apolipoprotein Al, Serum Amyloid P, Growth Hormone, Beta-2 microglobulin, Lipoprotein (a), MMP-9, Thyroid Stimulating hormone, alpha-2 Macroglobulin, Complement 3, IL-7, Leptin, and IL-6. Kits containing reagents to assist in the analysis of fluid samples are also described.

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METHODS AND KITS FOR THE DIAGNOSIS OF STROKE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Provisional Application No. 60/793,634, filed April 21, 2006, the disclosure of which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

[0002] The present invention relates to methods, kits and reagents for detection and/or diagnosis of stroke.

[0003] Every year, about 700,000 Americans suffer a stroke of which over 157,000 people a year die, making stroke the leading cause of morbidity and the number three cause of death in the United States behind cardiovascular diseases and cancer. Many victims succumb to a massive stroke and never make it to the emergency room. The more fortunate sufferers might complain of sudden loss of consciousness, cognitive deficits, speech dysfunction, limb weakness, hemiplegia, vertigo, diplopia, lower cranial nerve dysfunction, gaze deviation, ataxia, hemianopia and/or aphasia before being rushed to a hospital. The symptoms of stroke are by no means uniform among stroke sufferers.

[0004] Once suspected of suffering from stroke (a.k.a. transient ischemic attack), patients may be subjected to a battery of tests, including physical and neurological examination, blood *flow* tests and/or CT scans. However, notwithstanding the swift lethality of stroke, proper diagnosis of the condition remains inaccurate, cumbersome and often time consuming. What is more, current diagnostic methods to identify candidates at greatest risk to suffer a stroke before suffering one are not always preferable. Thus there exists a need for improving the screening and diagnosis of patients at risk for stroke.

[0005] Compounding the problem of providing adequate attention to stroke victims is the fact that the overall incidence of stroke is expected to rise as our population ages. Primary and secondary prevention of stroke is important to decrease its

incidence and its associated morbidity. Thus, there is a need for selective and sensitive assays for stroke. Similarly, there is a critical need to develop additional biomarkers for early detection and accurate diagnosis of stroke.

SUMMARY OF THE INVENTION

[0006] A method for rapid detection and/or accurate diagnosis of stroke is provided. The method can be practiced with a determination of the concentrations of one or two biomarkers in a patient fluid sample. Elevated (or depressed, as the case might be) levels of the one or two biomarkers, which are statistically different from levels found in “normals” (that is, control subjects not suffering from stroke), support a positive diagnosis of stroke. Preferably, the method utilizes a panel of analytes or “biomarkers,” up to twelve or more substances found in a sample fluid (*e.g.*, whole blood, serum, plasma, or urine), to help support a positive or negative diagnosis of stroke. Up to 99% accuracy in making a correct diagnosis may be provided by the method.

[0007] According to the invention a method of diagnosing stroke in a human subject suspected of suffering from stroke is provided, which comprises: (a) obtaining a fluid sample from a human subject suspected of suffering from stroke; (b) determining the concentration of Matrix Metallo Proteinase 3 (MMP-3) in said fluid sample; (c) deciding if the determined concentration of MMP-3 in said fluid sample is statistically different from that found in a control group of human subjects, whereby a statistically different elevated concentration of MMP-3 supports a positive diagnosis of stroke. Typically, the human subject is complaining of sudden weakness of the face, arm or leg. Any one of a number of fluid samples can be tested. Preferably, the fluid sample is selected from whole blood, plasma, serum, or urine. It has been discovered that a measured concentration of about 1 ng/mL or above of MMP-3 in the fluid sample supports a positive diagnosis of stroke.

[0008] In another aspect of the invention a method is provided for diagnosing stroke in a human subject suspected of suffering from stroke, which method comprises: (a) obtaining a fluid sample from a human subject suspected of suffering from stroke; (b) determining the concentration of Serum Glutamic Oxaloacetic Transaminase (SGOT)

in said fluid sample; (c) deciding if the determined concentration of SGOT in said fluid sample is statistically different from that found in a control group of human subjects, whereby a statistically different depressed concentration of SGOT supports a positive diagnosis of stroke. It is expected that a measured concentration of about 10 μg/mL or below of SGOT in said fluid sample will support a positive diagnosis of stroke.

[0009] Still another aspect of the invention relates to a method of diagnosing stroke in a human subject suspected of suffering from stroke, comprising: (a) obtaining a fluid sample from a human subject suspected of suffering from stroke; (b) determining the concentrations of MMP-3 and SGOT in said fluid sample; and (c) deciding if the determined concentrations of MMP-3 and SGOT in said fluid sample are statistically different from that found in a control group of human subjects, whereby a statistically different elevated concentration of MMP-3 and a statistically different depressed concentration of SGOT together support a positive diagnosis of stroke.

[0010] In a preferred embodiment, the method of the invention further comprises determining the concentration in said fluid sample of at least one of IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, TIMP-1, Ferritin, or Glutathione S-Transferase, or any combination thereof. Statistically elevated concentrations, compared to control levels, of all analytes mentioned above except SGOT, support a positive diagnosis of stroke. In particular, certain threshold levels of analytes in the sample fluids may be important in the detection or diagnosis of stroke, including IL-18 (about 300 pg/mL or above), Factor VII (about 320 ng/mL or above), ICAM-1 (about 170 ng/mL or above), Creatine Kinase-MB (about 5 ng/mL or above), MCP-1 (about 275 pg/mL or above), Myoglobin (about 30 ng/mL or above), C Reactive Protein (about 11 μg/mL or above), TIMP-1 (about 120 ng/mL or above), Ferritin (about 300 ng/mL or above), and Glutathione S-Transferase (about 2 ng/mL or above.

[0011] Yet other biomarkers may also be useful in arriving at a positive or negative diagnosis of stroke. These biomarkers include, in addition to those already disclosed, Prostate Specific Antigen (free), IL-3, Tissue Factor, alpha-Fetoprotein, Prostatic

Acid Phosphatase, Stem Cell Factor, MIP-1-beta, Carcinoembryonic Antigen, IL-13, TNF-alpha, IgE, Fatty Acid Binding Protein, ENA-78, IL-1-beta, Brain-Derived Nerotrophic Factor, Apolipoprotein A1, Serum Amyloid P, Growth Hormone, Beta-2 microglobulin, Lipoprotein (a), MMP-9, Thyroid Stimulating hormone, alpha-2 Macroglobulin, Complement 3, IL-7, Leptin, and IL-6.

[0012] Various techniques for assessing the importance of certain biomarkers in arriving at a diagnosis is also described herein. One such technique is a projection of compiled results on a proximity map, whereby the proximity of a subject's determined concentrations to a cluster of other subjects' determined concentrations, who were previously diagnosed as having suffered from stroke, contributes to a positive diagnosis of stroke. Other techniques include the application of one or more statistical methods (*e.g.*, linear regression analysis, classification tree analysis, heuristic nave Bayes analysis and the like).

[0013] Also provided is a kit comprising reagents for determining the concentration in a fluid sample of a panel of analytes including MMP-3, SGOT and one or more of IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, TIMP-1, Ferritin, or Glutathione S-Transferase. The reagents may include antibodies against the members of a given panel of analytes. Furthermore, the reagent may be immobilized on a substrate, which substrate may comprise a two-dimensional array, a microtiter plate, or multiple bead sets.

[0014] The methods may further comprise comparing the levels of the one, two, or more biomarkers in a patient's blood with levels of the same biomarkers in one or more control samples by applying a statistical method such as: linear regression analysis, classification tree analysis and heuristic nave Bayes analysis. The statistical method may be, and typically is performed by a computer process, such as by commercially available statistical analysis software. In one embodiment, the statistical method is a classification tree analysis, for example CART (Classification and Regression Tree). Results for a particular patient or subject, whose sample fluid is tested against a panel of biomarkers according to the method, can be projected onto a proximity map. The proximity of a particular patient's biomarker concentration results to one of at least two populations (those previously diagnosed as having

suffered a stroke and normals) supports a either a positive or negative diagnosis of stroke.

[0015] An article of manufacture is provided which comprises binding reagents specific for at least one of MMP-3 and SGOT, preferably both biomarkers. More preferably, a kit is provided which comprises binding reagents specific for MMP-3, SGOT, IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, TIMP-1, Ferritin, or Glutathione S-Transferase. In a preferred embodiment, each binding reagent is immobilized on a substrate. For example, monoclonal antibodies against MMP-3, SGOT and the other biomarkers described herein are immobilized independently to one or more discrete locations on one or more surfaces of one or more substrates. The substrates may be beads comprising an identifiable biomarker, wherein each binding reagent is attached to a bead comprising a different identifiable biomarker than beads to which a different binding reagent is attached. The identifiable biomarker may comprise a fluorescent compound, a quantum dot, or the like.

[0016] In another embodiment, a method is provided for determining the occurrence of a stroke in a patient, comprising determining levels of at least one of MMP-3 and SGOT.

[0017] In a further embodiment, a method of predicting onset of stroke is provided, comprising determining the change in concentration at two or more points in time of two or more markers in a patient's blood, wherein an observed increase in the concentration of MMP-3, a decrease in the concentration of SGOT or both, in the patient's blood between the two time points, is predictive of the onset of stoke.

In yet a further embodiment, a method of diagnosing stroke in a human subject suspected of suffering from stroke is provided, comprising: (a) obtaining a fluid sample from a human subject suspected of suffering from stroke; (b) determining the concentration of MMP-3 or SGOT or both in said fluid sample; (c) deciding if the determined concentration of MMP-3 or SGOT or both, respectively, in said fluid sample is statistically different from that found in a control group of human subjects, whereby a statistically different elevated concentration of MMP-3 or SGOT or both, respectively, supports a positive diagnosis of stroke. It has been discovered that a

measured concentration of about 1 ng/mL or above of MMP-3 or 10 μg/mL or below for SGOT in the fluid sample supports a positive diagnosis of stroke.

[0018] Other aspects of the invention will become apparent to those of ordinary skill after considering the detailed descriptions provided herewith.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIG. 1 is an example of a projection of a proximity map of patients whose fluid samples are tested against a panel of biomarkers listed on the right-hand margin. The results of this proximity map analysis indicate that consideration of all the biomarkers listed provides a degree of accuracy of a correct diagnosis of stroke of about 98%, with subjects having suffered a stroke positioned on the left-hand side of the figure (red or light gray dots) and subjects who have not suffered a stroke positioned on the right-hand side of the figure (blue or dark gray spots).

[0020] FIG. 2 provides an example of a proximity map of patients whose fluid samples have been tested against a panel of biomarkers listed on the right-hand margin. The results of this proximity map analysis would indicate that consideration of all the biomarkers listed provides a degree of accuracy of a correct diagnosis of stroke of about 97%, with subjects having suffered a stroke positioned on the left-hand side of the figure (red or light gray dots) and subjects who have not suffered a stroke positioned on the right-hand side of the figure (blue or dark gray spots).

[0021] FIG. 3 is another example of a proximity map of patients whose fluid samples have been tested against a panel of biomarkers listed on the right-hand margin (except that the results for the biomarkers Tissue Factor and vWF are excluded from this analysis). The results of this proximity map analysis indicate that consideration of all the biomarkers listed provides a degree of accuracy of a correct diagnosis of stroke of about 99%, with subjects having suffered a stroke positioned on the left-hand side of the figure (red or light gray dots) and subjects who have not suffered a stroke positioned on the right-hand side of the figure (blue or dark gray spots).

[0022] FIG. 4 is yet another example of a proximity map of patients whose fluid samples are tested against a panel of biomarkers listed on the right-hand margin. The

results of this proximity map analysis would indicate that consideration of all the biomarkers listed provides a degree of accuracy of a correct diagnosis of stroke of about 94%, with subjects having suffered a stroke positioned on the left-hand side of the figure (red or light gray dots) and subjects who have not suffered a stroke positioned on the right-hand side of the figure (blue or dark gray spots).

[0023] FIG. 5 is still another example of a proximity map of patients whose fluid samples were tested against a panel of biomarkers listed on the right-hand margin. The results of this proximity map analysis suggest that consideration of all the biomarkers listed provides a degree of accuracy of a correct diagnosis of stroke of about 97%, with subjects having suffered a stroke positioned on the left-hand side of the figure (red or light gray dots) and subjects who have not suffered a stroke positioned on the right-hand side of the figure (blue or dark gray spots).

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0024] Stroke, as defined herein, broadly refers as the sudden deficit in brain function usually related to impaired cerebral blood flow. Strokes may be further defined by the nature of the deficiency (*e.g.*, thrombosis, embolism, hemorrhage, ischemia). However, solely in the interest of clarity in drafting, the term “stroke” is meant to encompass any deficiency in brain function related to impaired cerebral blood flow, and not limited to a particular source for said impairment.

[0025] The use of numerical values in the various ranges specified in this application, unless expressly indicated otherwise, are stated as approximations as though the minimum and maximum values within the stated ranges were both preceded by the word “about.” In this manner, slight variations above and below the stated ranges can be used to achieve substantially the same results as values within the ranges. Also, the disclosure of these ranges is intended as a continuous range including every value between the minimum and maximum values.

[0026] Provided herein is a multifactorial assay for rapid identification of a stroke patient. Identified below are certain sample fluid (*e.g.*, blood) analytes or biomarkers useful in the detection and/or diagnosis of stroke. It has been found that the following biomarkers may be over-expressed in the blood of patients suffering from or who

have suffered a stroke. SGOT, for example, may be under-expressed in patients suffering from or who have suffered a stroke.

[0027] Also identified as being useful in the detection or proper diagnosis of subjects suffering from or who have suffered a stroke are the biomarkers MMP-3, IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, von Willebrand Factor, TIMP-1, Ferritin, Glutathione S-Transferase, Prostate Specific Antigen (free), IL-3, Tissue Factor, alpha-Fetoprotein, Prostatic Acid Phosphatase, Stem Cell Factor, MIP-1-beta, Carcinoembryonic Antigen, IL-13, TNF-alpha, IgE, Fatty Acid Binding Protein, ENA-78, IL-1-beta, Brain-Derived Nerotrophic Factor, Apolipoprotein A1, Serum Amyloid P, Growth Hormone, Beta-2 microglobulin, Lipoprotein (a), MMP-9, Thyroid Stimulating hormone, alpha-2 Macroglobulin, Complement 3, IL-7, Leptin, and/or IL-6.

[0028] The parameters for establishing the significance of one or more biomarkers for the diagnosis of stroke are determined statistically by comparing normal or control blood (preferably, *e.g.*, serum or plasma) levels of these biomarkers with blood levels in patients clinically and properly diagnosed as having suffered from or is having a stroke. The statistical data presented below in Table 1 identify certain mean values and accompanying standard deviations of the above-described biomarkers, which may be found in the blood of stroke patients in comparison to normals. Accordingly, as a non-limiting example of estimates of significant threshold values, which can support of a positive diagnosis of stroke, the following concentrations are provided: MMP-3 (about 1 ng/mL or above), SGOT (about 10 μg/mL or below), IL-18 (about 300 pg/mL or above), Factor VII (about 320 ng/mL or above), ICAM-1 (about 170 ng/mL or above), Creatine Kinase-MB (about 5 ng/mL or above), MCP-1 (about 275 pg/mL or above), Myoglobin (about 30 ng/mL or above), C Reactive Protein (about 11 μg/mL or above), TIMP-1 (about 120 ng/mL or above), Ferritin (about 300 ng/mL or above), or Glutathione S-Transferase (about 2 ng/mL or above).

[0029] It is understood that these values are approximate. Statistical methods can be used to define the critical range of values. Typically within one standard deviation of those approximate values might be considered as statistically significant values for determining a statistically significant difference, preferably two standard deviations.

For this reason, the word “about” is used in connection with the stated values. “Statistical classification methods” are used to identify biomarkers capable of discriminating normal patients from patients with stroke and are further used to determine critical blood values for each biomarker for discriminating between such patients.

[0030] Certain statistical methods can be used to identify discriminating biomarkers and panels thereof. These statistical methods may include, but are not limited to: 1) linear regression; 2) classification tree methods; and 3) statistical machine learning to optimize the unbiased performance of algorithms for making predictions. Each of these statistical methods is well-known to those of ordinary skill in the field of biostatistics and can be performed as a process in a computer. A large number of software products are available commercially to implement statistical methods, such as, without limitation, S-PLUS™, commercially available from Insightful Corporation of Seattle, Washington; however, the instant invention is not limited to the use of any one specific software.

[0031] By identifying biomarkers useful in the determination and/or diagnosis of stroke and by use of statistical methods to identify which biomarkers and groups of biomarkers are particularly useful in identifying stroke-at-risk patients, a person of ordinary skill in the art, based on the disclosure herein, can compose panels of biomarkers having superior selectivity and sensitivity. Examples of biomarkers that can be included in panels, which provide excellent discriminatory capability, include: MMP-3, SGOT, IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, von Willebrand Factor, TIMP-1, Ferritin, Glutathione S-Transferase, Prostate Specific Antigen (free), IL-3, Tissue Factor, alpha-Fetoprotein, Prostatic Acid Phosphatase, Stem Cell Factor, MIP-1-beta, Carcinoembryonic Antigen, IL-13, TNF-alpha, IgE, Fatty Acid Binding Protein, ENA-78, IL-1-beta, Brain-Derived Nerotrophic Factor, Apolipoprotein A1, Serum Amyloid P, Growth Hormone, Beta-2 microglobulin, Lipoprotein (a), MMP-9, Thyroid Stimulating hormone, alpha-2 Macroglobulin, Complement 3, IL-7, Leptin, and IL-6. Examples of specific panels comprising selected biomarkers from the above-mentioned list, include, but are not limited to: (i) CRP, CKMB, Factor VII,

Ferritin, GST, ICAM-1, IL-18, IL-1B, IL-3, MCP-1, MMP-3, Myoglobin, SGOT, TIMP-1 and vWF; (ii) CRP, CKMB, Factor VII, Ferritin, GST, ICAM-1, IL-18, MCP-1, MMP-3, Myoglobin, SGOT, TIMP-1, Tissue Factor and vWF; (iii) CRP, CKMB, Factor VII, Ferritin, GST, ICAM-1, IL-18, MCP-1, MMP-3, Myoglobin, SGOT and TIMP-1; (iv) SGOT, CKMB, MMP-3, GST, Factor VII, IL-18, IL-3, MCP-1, ICAM-1 and IL-1B; (v) SGOT, CKMB, MMP-3, GST and Factor VII; (vi) SGOT and MMP-3. It will be recognized by those of ordinary skill in the field of biostatistics, that the number of biomarkers in any given panel may be different depending on the combination of biomarkers. With optimum sensitivity and specificity being the goal, one panel may include two biomarkers, another may include five, and still others may include twelve or more, yielding similar results.

[0032] The invention is based on an evaluation of at least MMP-3 levels, alone or in combination with levels of immunological SGOT and/or other biomarkers, in serum for diagnosis of stroke in all stages of their occurrence. The invention is also based on the evaluation of at least immunological SGOT levels, optionally in combination with levels of at least MMP-3. Patients with stroke are at considerable risk for death and serious complications, and outcomes can be improved with appropriate diagnosis and therapy. Thus, rapid and accurate diagnosis of patients complaining of the symptoms of stroke is critical for patient care.

[0033] The results described herein suggest that serum MMP-3 levels are elevated in stroke. Thus, MMP-3 can be used as an early biomarker of inflammatory cardiovascular conditions, including stroke. By the same token, SGOT levels are appear depressed in stroke patients. Thus, SGOT can also be used as an early biomarker of stroke.

[0034] The present method includes measuring the level of MMP-3 and/or SGOT in a biological sample (*e.g.*, whole blood, plasma, serum or urine and the like) from a patient; comparing the respective levels with that of control subjects; and diagnosing the state of disease based on the level of MMP-3 or SGOT relative to that of control subjects. In this way, a patient can be diagnosed with stroke if the level of MMP-3 is increased relative to that of control subjects or if SGOT is decreased relative to controls.

[0035] A typical control value for MMP-3 is in the range of about 0.1–0.8 ng/mL. A concentration of about 1 ng/mL or above in a patient sample can support a positive diagnosis. The general range for elevated values of MMP-3 is about 1.5–20 ng/mL.

[0036] A typical control value for SGOT is in the range of about 17–25 μg/mL. An immunological concentration of about 10 μg/mL or below in a patient sample can support a positive diagnosis. SGOT is often measured enzymatically. However, here, the sum of protein is presented, which may include enzymatically inactive plus enzymatically active SGOT. The general range for depressed values of immunological SGOT concentration is about 1–15 μg/mL.

[0037] MMP-3 and SGOT can be captured with anti-MMP-3 and anti-SGOT polyclonal antibodies, respectively, or with corresponding monoclonal antibodies. The diagnostic method may also include measuring the levels of one or more additional analytes selected from the group consisting of: IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, von Willebrand Factor, TIMP-1, Ferritin, Glutathione S-Transferase, Prostate Specific Antigen (free), IL-3, Tissue Factor, alpha-Fetoprotein, Prostatic Acid Phosphatase, Stem Cell Factor, MIP-1-beta, Carcinoembryonic Antigen, IL-13, TNF-alpha, IgE, Fatty Acid Binding Protein, ENA-78, IL-1-beta, Brain-Derived Nerotrophic Factor, Apolipoprotein A1, Serum Amyloid P, Growth Hormone, Beta-2 microglobulin, Lipoprotein (a), MMP-9, Thyroid Stimulating hormone, alpha-2 Macroglobulin, Complement 3, IL-7, Leptin, and IL-6; and diagnosing the patient's condition based on the level of the additional analyte and the level of MMP-3 and/or SGOT relative to that of control subjects.

[0038] Analyte levels can be measured using an immunoassay such as an ELISA or a multiplexed method as described below, and in more detail by Chandler et al., U.S. 5,981,180 (Luminex Corporation).

[0039] MMP-3 levels above about 1 ng/mL has been identified in unstable angina patients and myocardial infarction patients. SGOT levels below 10 μg/mL were also identified in unstable angina patients and myocardial infarction patients. Both biomarkers are associated with unfavorable outcomes when elevated. Indeed, MMP-3 is a valuable unstable plaque biomarker even when troponins and C-reactive protein are not elevated, potentially identifying high-risk patients who otherwise might

remain undiagnosed. Without being bound by a particular mechanism, MMP-3 may be directly involved in the pathophysiology of stroke as well. The role SGOT plays in the pathophysiology of stroke is also not known; however, the same maker can be used to diagnose the disease.

[0040] The analytes used in the method of the invention can be detected, for example, by a binding assay. For example, a sandwich immunoassay can be performed by capturing MMP-3 and SGOT from a biological sample with antibodies having specific binding affinity for each protein, which then can be detected with a labeled antibody having specific binding affinity for each analyte. Alternatively, standard immunohistochemical techniques can be used to detect MMP-3 and SGOT using such antibodies. Antibodies having affinity for MMP-3 and SGOT are available.

[0041] The term "binding reagent" and like terms, refers to any compound, composition or molecule capable of specifically or substantially specifically (that is with limited cross-reactivity) binding another compound or molecule, which, in the case of immune-recognition is an epitope. The binding reagents typically are antibodies, preferably monoclonal antibodies, or derivatives or analogs thereof, but also include, without limitation: Fv fragments; single chain Fv (scFv) fragments; Fab' fragments; F(ab')2 fragments; humanized antibodies and antibody fragments; camelized antibodies and antibody fragments; and multivalent versions of the foregoing. Multivalent binding reagents also may be used, as appropriate, including without limitation: monospecific or bispecific antibodies, such as disulfide stabilized Fv fragments, scFv tandems ((scFv)2 fragments), diabodies, tribodies or tetrabodies, which typically are covalently linked or otherwise stabilized (*i.e.*, leucine zipper or helix stabilized) scFv fragments. "Binding reagents" also include aptamers, as are described in the art.

[0042] Methods of making antigen-specific binding reagents, including antibodies and their derivatives and analogs and aptamers, are well-known in the art. Polyclonal antibodies can be generated by immunization of an animal. Monoclonal antibodies can be prepared according to standard (hybridoma) methodology. Antibody derivatives and analogs, including humanized antibodies can be prepared

recombinantly by isolating a DNA fragment from DNA encoding a monoclonal antibody and subcloning the appropriate V regions into an appropriate expression vector according to standard methods. Phage display and aptamer technology is described in the literature and permit in vitro clonal amplification of antigen-specific binding reagents with very affinity low cross-reactivity. Phage display reagents and systems are available commercially, and include Aptamer technology described for example and without limitation in U.S. Pat. Nos. 5,270,163, 5,475096, 5,840867 and 6,544,776.

[0043] The ELISA and Luminex xMAP immunoassays described below are examples of sandwich assays. The term "sandwich assay" refers to an immunoassay where the antigen is sandwiched between two binding reagents, which are typically antibodies. The first binding reagent/antibody being attached to a surface and the second binding reagent/antibody comprising a detectable group. Examples of detectable groups include, for example and without limitation: fluorochromes, enzymes, epitopes for binding a second binding reagent (for example, when the second binding reagent/antibody is a mouse antibody, which is detected by a fluorescently-labeled anti-mouse antibody), for example an antigen or a member of a binding pair, such as biotin. The surface may be a planar surface, such as in the case of a typical grid-type array (for example, but without limitation, 96-well plates and planar microarrays), as described herein, or a non-planar surface, as with coated bead array technologies, where each "species" of bead is labeled with, for example, a fluorochrome (such as the Luminex technology described herein and in U.S. Pat. Nos. 6,599,331, 6,592,822 and 6,268,222), or quantum dot technology (for example, as described in U.S. Pat. No. 6,306,610).

[0044] In the bead-type immunoassays described in the examples below, the Luminex xMAP system may be utilized. The xMAP system incorporates polystyrene microspheres that are dyed internally with two spectrally distinct fluorochromes. Using precise ratios of these fluorochromes, an array is created consisting of 100 different microsphere sets with specific spectral addresses. Each microsphere set can possess a different reactant on its surface. Because microsphere sets can be distinguished by their spectral addresses, they can be combined, allowing up to 100

different analytes to be measured simultaneously in a single reaction vessel. A third fluorochrome coupled to a reporter molecule quantifies the biomolecular interaction that has occurred at the microsphere surface. Microspheres are interrogated individually in a rapidly flowing fluid stream as they pass by two separate lasers in the Luminex analyzer. High-speed digital signal processing classifies the microsphere based on its spectral address and quantifies the reaction on the surface in a few seconds per sample.

[0045] For the assays described herein, the bead-type immunoassays are preferable for a number of reasons. As compared to ELISAs, costs and throughput are far superior. As compared to typical planar antibody microarray technology the beads are superior for quantitation purposes because the bead technology does not require pre-processing or titering of the plasma or serum sample, with its inherent difficulties in reproducibility, cost and technician time. For this reason, although other immunoassays, such as, without limitation, ELISA, RIA and antibody microarray technologies, are capable of use in the context of the present invention, but they are not preferred. As used herein, “immunoassays” refer to immune assays, typically, but not exclusively sandwich assays, capable of detecting and quantifying a desired blood biomarker, namely at least one of MMP-3, SGOT, IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, TIMP-1, Ferritin and Glutathione S-Transferase, or any combination of the foregoing.

[0046] Data generated from an assay to determine blood levels of one, two, three, or four or more of the biomarkers MMP-3, SGOT, IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, von Willebrand Factor, TIMP-1, Ferritin, Glutathione S-Transferase, Prostate Specific Antigen (free), IL-3, Tissue Factor, alpha-Fetoprotein, Prostatic Acid Phosphatase, Stem Cell Factor, MIP-1-beta, Carcinoembryonic Antigen, IL-13, TNF-alpha, IgE, Fatty Acid Binding Protein, ENA-78, IL-1-beta, Brain-Derived Nerotrophic Factor, Apolipoprotein A1, Serum Amyloid P, Growth Hormone, Beta-2 microglobulin, Lipoprotein (a), MMP-9, Thyroid Stimulating hormone, alpha-2 Macroglobulin, Complement 3, IL-7, Leptin and IL-6 can be used to determine the likelihood of a patient suffering a stroke.

[0047] As shown herein, if any one or more, two or more, typically three or four or more of the following conditions are met in a patient's blood—MMP-3 (about 1 ng/mL or above), SGOT (about 10 μg/mL or below), IL-18 (about 300 pg/mL or above), Factor VII (about 320 ng/mL or above), ICAM-1 (about 170 ng/mL or above), Creatine Kinase-MB (about 5 ng/mL or above), MCP-1 (about 275 pg/mL or above), Myoglobin (about 30 ng/mL or above), C Reactive Protein (about 11 μg/mL or above), TIMP-1 (about 120 ng/mL or above), Ferritin (about 300 ng/mL or above), or Glutathione S-Transferase (about 2 ng/mL or above)—there is a very high likelihood that the patient has suffered or is suffering from stroke. In one embodiment, either an elevated MMP-3 level or a depressed SGOT level alone, relative to the level of the biomarker of interest in a population of normal or control patients, indicates the existence of stroke in the patient with, preferably, about a 97-99% level of certainty. (See, Table 2, discussed further herein.)

[0048] In the context of the present disclosure, "blood" includes any blood fraction, for example plasma, that can be analyzed according to the methods described herein. Serum is a standard blood fraction that can be tested, and is tested in the Examples below. By measuring blood levels of a particular biomarker, it is meant that any appropriate blood fraction can be tested to determine blood levels and that data can be reported as a value present in that fraction. As a non-limiting example, the blood levels of a biomarker can be presented as 50 pg/mL serum.

[0049] As described above, methods for diagnosing stroke by determining levels of specific identified blood biomarkers are provided. Also provided are methods of detecting preclinical stroke comprising determining the presence and/or velocity of specific identified biomarkers in a patient's blood. By velocity it is meant the changes in the concentration of the biomarker in a patient's blood over time. Longitudinal data has value in determining the velocity of specific biomarkers in a patient's blood for predicting the onset of clinical stroke. Biomarkers with velocity indicative of preclinical stroke, include: MMP-3, IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, von Willebrand Factor, TIMP-1, Ferritin, Glutathione S-Transferase, Prostate Specific Antigen (free), IL-3, Tissue Factor, alpha-Fetoprotein, Prostatic Acid Phosphatase, Stem Cell Factor, MIP-

1-beta, Carcinoembryonic Antigen, IL-13, TNF-alpha, IgE, Fatty Acid Binding Protein, ENA-78, IL-1-beta, Brain-Derived Nerotrophic Factor, Apolipoprotein A1, Serum Amyloid P, Growth Hormone, Beta-2 microglobulin, Lipoprotein (a), MMP-9, Thyroid Stimulating hormone, alpha-2 Macroglobulin, Complement 3, IL-7, Leptin and IL-6 which increase in concentration beginning at about 1-24 months prior to clinical onset of stroke; and SGOT which decreases in concentration beginning at 1-24 months prior to clinical onset of stroke.

EXAMPLE I

[0050] Patient Population. In one study, a patient population is chosen based on a strong predisposition to stroke indicated by such factors as age, diet, exercise, medical history and familial background; or suffering from stroke as indicated by CT scans or MRI. A normal or control patient population can be chosen from a wellness clinic. These control patients have no indication of suffering from cardiovascular disease. Consent and blood specimens from all participants should be obtained under IRB Protocol.

[0051] Collection and storage of blood specimens: 10 mL of peripheral blood may be drawn from subjects using standardized phlebotomy procedures. Blood samples are then collected without anticoagulant into two 5 mL red top vacutainers, the sera separated by centrifugation, and all specimens immediately frozen and stored in the dedicated –80 °C freezer.

[0052] All blood samples can be logged on the study computer to track information such as storage date, freeze/thaw cycles and distribution.

[0053] Development of Luminex assay. The reagents for multiplex system may be developed using commercially available antibody or produced by well known immunological methods. Capture antibodies are generally monoclonal and detection antibodies polyclonal. Capture Abs are covalently coupled to carboxylated polystyrene microspheres (Luminex Corporation (Austin, Tex.)). Covalent coupling of the capture antibodies to the microspheres is preferably performed by following the procedures recommended by Luminex. In short, the microspheres' stock solutions are dispersed in a sonification bath for 2 min. An aliquot of 2.5×10^6 microspheres is

resuspended in microtiter tubes containing 0.1 M sodium phosphate buffer, pH 6.1 (phosphate buffer), to a final volume of 80 μL.

[0054] This suspension is then sonicated until a homogeneous distribution of the microspheres is observed. Solutions of N-hydroxy-sulfosuccinimide (Sulfo-NHS) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride, both at 50 mg/mL, are prepared in phosphate buffer, and 10 μL of each solution is sequentially added to stabilize the reaction and activate the microspheres. This suspension is subsequently incubated for 10 min at room temperature and then resuspended in 250 μL of PBS containing 50 μg of antibody. The mixture is incubated overnight in the dark with continuous shaking. Microspheres are then incubated with 250 μL of PBS-0.05% Tween 20 for 4 h. After aspiration, the beads are blocked with 1 mL of PBS-1% BSA-0.1% sodium azide. The microspheres are counted with a hemacytometer and stored at a final concentration of 10^6 microspheres per mL in the dark at 4 °C. Coupling efficiency of monoclonal antibodies can be tested by staining about 2,000 microspheres with PE-conjugated goat anti-mouse IgG. Detection Abs are biotinylated and the extent of biotin incorporation, which may be determined using HABA assay, was 20 moles of biotin per mole of protein. The assays may be further optimized for concentration of detection Ab and for incubation times.

[0055] Examples of some commercial sources of matched antibody cytokine pairs include R&D Systems, Minneapolis, MN and Fitzgerald Industries International, Inc., Concord, MA.

[0056] Sensitivity of the newly developed assay, determined in a Luminex assay using serially diluted purified CA-125, is typically 20 IU. Intra-assay variability, expressed as a coefficient of variation, is preferably calculated based on the average for patient samples and measured twice at least two different time points. The intra-assay variability within the replicates presented as an average coefficient of variation is generally about 8.5%.

[0057] Statistical Analysis of Data. All statistical analyses may be conducted using S-Plus statistical software (Seattle, Wash.: Math Soft, Inc., 1999). The data are first randomly split into a training and test set. Logistic regression is then used to calculate the optimal weighting of each biomarker and the subsequent predicted probability of

being a case. All predicted probabilities ≥0.5 are categorized as a predicted case; predicted probabilities <0.5 are categorized as a predicted control. After fitting a logistic model to the training set, classification of disease status is then calculated for the test set.

RESULTS

[0058] Serum concentrations of biomarkers by xMap technology. Circulating concentrations of different serum biomarkers are evaluated in a multiplexed assay using xMap technology in blood of patients from stroke and control groups. Table 1 lists the analytes that can be statistically different between the two groups.

TABLE 1

Significant Analytes in Stroke Patients

	Units	stroke Mean	stroke SD	Control Mean	Control SD	P value (t-test)
IL-18	pg/mL	378	184	128	88	8.7E-22
Factor VII	ng/mL	523	170	205	58	1.7E-21
SGOT	ug/mL	5.7	9.8	21	4.4	6.3E-21
ICAM-1	ng/mL	216	92	108	31	2.0E-19
Creatine Kinase-MB	ng/mL	32	25	0.69	0.70	2.0E-19
MCP-1	pg/mL	349	159	151	124	9.4E-15
Myoglobin	ng/mL	73	71	16	7.8	2.8E-13
MMP-3	ng/mL	10	8.5	0.41	0.31	4.9E-11
C Reactive Protein	ug/mL	17	19	3.4	4.1	9.5E-11
von Willebrand Factor	ug/mL	25	14	12	9.4	2.2E-10
TIMP-1	ng/mL	136	50	100	18	1.6E-09
Ferritin	ng/mL	355	292	178	124	1.2E-07
Glutathione S-Transferase	ng/mL	22	25	0.62	0.55	1.5E-07
Prostate Specific Antigen, Free	ng/mL	0.47	0.35	0.20	0.24	3.5E-07
IL-3	ng/mL	0.46	0.28	0.087	0.059	8.2E-07
Tissue Factor	ng/mL	5.4	3.6	2.9	2.1	2.0E-06
Alpha-Fetoprotein	ng/mL	7.5	3.7	4.7	2.7	3.6E-06
Prostatic Acid Phosphatase	ng/mL	0.41	0.30	0.24	0.15	6.4E-06
Stem Cell Factor	pg/mL	98	54	44	37	3.3E-05
MIP-1beta	pg/mL	147	159	79	52	7.5E-05

Carcinoembryonic Antigen	ng/mL	3.5	4.4	1.7	1.3	1.3E-04
IL-13	pg/mL	57	35	41	14	1.9E-04
TNF-alpha	pg/mL	17	27	7.3	5.7	6.3E-04
IgE	ng/mL	260	327	108	161	1.4E-03
Fatty Acid Binding Protein	ng/mL	20	26	6.6	6.7	1.5E-03
ENA-78	ng/mL	1.2	1.3	0.64	0.70	1.9E-03
IL-1beta	pg/mL	7.0	6.9	4.0	3.1	2.2E-03
Brain-Derived Neurotrophic Factor	ng/mL	3.6	4.7	2.2	1.8	3.2E-03
Apolipoprotein A1	mg/mL	0.68	0.48	0.84	0.21	4.0E-03
Serum Amyloid P	ug/mL	34	7.0	30	8.7	5.0E-03
Growth Hormone	ng/mL	1.5	1.5	0.72	1.4	5.2E-03
Beta-2 Microglobulin	ug/mL	2.3	0.98	2.0	0.55	5.7E-03
Lipoprotein (a)	ug/mL	99	112	52	84	7.5E-03
MMP-9	ng/mL	217	159	313	235	9.3E-03
Thyroid Stimulating Hormone	uIU/mL	2.1	1.4	1.5	1.1	1.0E-02
Alpha-2 Macroglobulin	mg/mL	0.39	0.65	0.23	0.078	1.0E-02
Complement 3	mg/mL	1.4	0.65	1.2	0.26	1.2E-02
IL-7	pg/mL	37	22	44	16	1.9E-02
Leptin	ng/mL	18	30	11	10	2.5E-02
IL-6	pg/mL	54	43	30	18	3.1E-02

[0059] Table 2 illustrates the diagnostic accuracy, which may be obtained by testing for each individual analyte and determining their usefulness as a diagnostic tool. The best individual biomarkers would appear to be MMP-3 and SGOT. The best combination of biomarkers would appear to be the twelve analytes that are shown in FIG. 4.

TABLE 2								
Analyte Accuracy for Stroke								
MMP-3	Neg	Pos	Accuracy		MCP-1	Neg	Pos	Accuracy
True Neg	89	0			True Neg	84	5	
True Pos	2	57	99%		True Pos	13	46	88%
SGOT	Neg	Pos	Accuracy		IL-18	Neg	Pos	Accuracy
True Neg	88	1			True Neg	82	7	

True Pos	3	56	97%		True Pos	12	47	87%
CKMB	Neg	Pos	Accuracy		Myoglobin	Neg	Pos	Accuracy
True Neg	86	3			True Neg	80	9	
True Pos	6	53	94%		True Pos	16	43	83%
GST	Neg	Pos	Accuracy		CRP	Neg	Pos	Accuracy
True Neg	85	4			True Neg	78	11	
True Pos	8	51	92%		True Pos	21	38	78%
Factor VII	Neg	Pos	Accuracy		Ferritin	Neg	Pos	Accuracy
True Neg	84	5			True Neg	79	10	
True Pos	8	51	91%		True Pos	25	34	76%
ICAM-1	Neg	Pos	Accuracy		TIMP-1	Neg	Pos	Accuracy
True Neg	82	7			True Neg	80	9	
True Pos	9	50	89%		True Pos	27	32	76%

[0060] Proximity Map Analysis. The proximity map data analysis can be conducted with a software program that groups samples by their similarities in analyte concentration patterns. A unique chemical signature is generated using the concentration of the analytes measured in each sample. The relationship of each sample signature is visualized in the Galaxy™ projection. The Galaxy™ is a proximity map, such that the closer two objects are in the visualization, the closer their chemical signatures are, and thus the more similar they are to one another. The axes are dimensionless (a result of being derived from a principal component analysis), and thus the visualization is not a typical X-Y scatter plot in which moving along one axis means increasing or decreasing a single value. The two axes of the Galaxy™ are defined by the first two principal components, a common method to reduce complex data. The placement of objects (record points) is done using a set of heuristics that have been designed to maximize the preservation of spatial relationships that existed in the high-dimensional space of the original data while minimizing the overlap that can occur when doing simple projections.

[0061] An examination of the FIGs, shows that the red circles (light gray, the stroke patients) are separated from the blue circles (dark gray, controls) to various degrees with all of the plots attaining fairly good separation. FIG. 3 provides what is possibly the best separation. If an unknown sample is tested for the analytes listed in FIG. 3, then the location of the patient (from whom the unknown sample is taken) on the plot would be indicative of whether that patient is having, in the instant invention, stroke or not. The space between the two clusters would appear to be an indeterminate area. In FIG. 3 there appears to be one stroke patient that lands in the middle of the control population. This results might be a false negative or the original clinical diagnosis might have been simply incorrect.

[0062] Rates of classification accuracy (in discriminating controls from stroke patients) can then be obtained using 10-fold cross-validation and generation of a Receiver Operating Characteristic (ROC) curve. The sensitivity and specificity of the method depend on the cut-point (*i.e.*, predicted probability from the classification tree) used to classify each subject as either a case or control. In the instant example, using the standard cut-point of 0.5 (*i.e.*, everyone with a predicted probability above 0.5 is classified as a cancer case) gives 100% sensitivity, 86% specificity, and 93% correctly classified. Fixing the specificity at 91% still leads to a very high sensitivity, at 95.5% (again with 93% correctly classified). Alternatively, a specificity of 95.3% corresponds to a sensitivity of 84.1% (and 90.0% correctly classified). The total area under the receiver operating characteristic (ROC) curve is near one (which would represent perfect classification), at 0.966.

EXAMPLE II

[0063] Development of xMAP Assays for Circulating Antibodies. Assays may be performed in filter-bottom 96-well microplates. Purified antigens of interest are coupled to Luminex beads as described for antibodies. Antigen-coupled beads are pre-incubated with blocking buffer containing 4% BSA for 1 h at room temperature on microtiter shaker. Beads are then washed three times with washing buffer (PBS, 1% BSA, 0.05% Tween 20) using a vacuum manifold followed by incubation with 50 μL blood serum diluted 1:250 for 30 min at 4 °C. This dilution is selected as an optimal for recovery of anti-IL-18 IgG based on previous serum titration. Next, the

washing procedure is repeated as above and beads are incubated with 50 μL/well of 4 μg/mL PE-conjugated antibody raised against human IgG, for 45 min in the dark with the constant shaking. Wells are washed twice, assay buffer added to each well, and samples analyzed using the xMap suspension array system. For a standard curve, antigen-coupled beads may be incubated with serially diluted human antibodies against specific antigens. Purification of monospecific human antibodies is described above. Data analysis may be performed using five-parametric-curve fitting.

[0064] Whereas particular embodiments of the invention have been described with respect to specific examples herein for the purpose of illustrating the invention and not for the purpose of limiting the same, it will be appreciated by those of ordinary skill in the art that numerous variations of the details, materials and arrangement of parts may be made within the principle and scope of the invention without departing from the invention as described in the appended claims.

WHAT IS CLAIMED IS:

1. A method of diagnosing stroke in a human subject suspected of suffering from stroke, comprising:

(a) obtaining a fluid sample from a human subject suspected of suffering from stroke;

(b) determining the concentration of MMP-3 in said fluid sample;

(c) deciding if the determined concentration of MMP-3 in said fluid sample is statistically different from that found in a control group of human subjects,

whereby a statistically different elevated concentration of MMP-3 supports a positive diagnosis of stroke.

2. The method of claim 1 in which said human subject is complaining of sudden weakness of the face, arm or leg, or other signs of stroke.

3. The method of claim 1 in which said fluid sample is selected from the group consisting of whole blood, plasma, serum, or urine.

4. The method of claim 1 in which a determined concentration of about 1 ng/mL or above of MMP-3 in said fluid sample supports a positive diagnosis.

5. A method of diagnosing stroke in a human subject suspected of suffering from stroke, comprising:

(a) obtaining a fluid sample from a human subject suspected of suffering from stroke;

(b) determining the concentration of SGOT in said fluid sample;

(c) deciding if the determined concentration of SGOT in said fluid sample is statistically different from that found in a control group of human subjects,

whereby a statistically different depressed concentration of SGOT supports a positive diagnosis of stroke.

6. The method of claim 5 in which said human subject is complaining of sudden weakness of the face, arm or leg, or other signs of stroke.

7. The method of claim 5 in which a determined concentration of about 10 μg/mL or below of SGOT in said fluid sample supports a positive diagnosis.

8. A method of diagnosing stroke in a human subject suspected of suffering from stroke, comprising:

(a) obtaining a fluid sample from a human subject suspected of suffering from stroke;

(b) determining the concentrations of MMP-3 and SGOT in said fluid sample;

(c) deciding if the determined concentrations of MMP-3 and SGOT in said fluid sample are statistically different from that found in a control group of human subjects,

whereby a statistically different elevated concentration of MMP-3 and a statistically different depressed concentration of SGOT support a positive diagnosis of stroke.

9. The method of claim 8 which further comprises determining the concentration in said fluid sample of at least one of IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, TIMP-1, Ferritin, or Glutathione S-Transferase, or any combination thereof.

10. The method of claim 9 in which statistically different elevated concentrations, compared to control levels, of all analytes except SGOT support a positive diagnosis of stroke.

11. The method of claim 9 in which concentrations are determined by conducting one or more immunoassays.

12. The method of claim 9 which further comprises determining the concentration in said fluid sample of at least one of IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, von Willebrand Factor,

TIMP-1, Ferritin, Glutathione S-Transferase, Prostate Specific Antigen (free), IL-3, Tissue Factor, alpha-Fetoprotein, Prostatic Acid Phosphatase, Stem Cell Factor, MIP-1-beta, Carcinoembryonic Antigen, IL-13, TNF-alpha, IgE, Fatty Acid Binding Protein, ENA-78, IL-1-beta, Brain-Derived Nerotrophic Factor, Apolipoprotein A1, Serum Amyloid P, Growth Hormone, Beta-2 microglobulin, Lipoprotein (a), MMP-9, Thyroid Stimulating hormone, alpha-2 Macroglobulin, Complement 3, IL-7, Leptin, or IL-6, or any combination thereof.

13. The method of claim 12 in which statistically different elevated concentrations, compared to control levels, of all analytes except SGOT support a positive diagnosis of stroke.

14. The method of claim 9 in which a subject's determined concentrations of analytes in said fluid sample are presented in a proximity map, whereby the proximity of a subject's determined concentrations to a cluster of other subjects' determined concentrations, who were previously diagnosed as having suffered from stroke, contributes to a positive diagnosis of stroke.

15. A kit comprising reagents for determining the concentration in a fluid sample of a panel of analytes including MMP-3, SGOT and one or more of IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, TIMP-1, Ferritin, or Glutathione S-Transferase.

16. The kit of claim 15 which includes antibodies against a panel of analytes including MMP-3, SGOT, IL-18, Factor VII, ICAM-1, Creatine Kinase-MB, MCP-1, Myoglobin, C Reactive Protein, TIMP-1, Ferritin, and Glutathione S-Transferase.

17. The kit of claim 15 in which the reagents are immobilized on a substrate.

18. The kit of claim 17 in which the substrate comprises a two-dimensional array, a microtiter plate, a strip, multiple bead sets, or any other method of performing an immunoassay.

19. The method of claim 8 which includes applying a statistical method selected from the group consisting of linear regression analysis, classification tree analysis and heuristic nave Bayes analysis.

20. The method of claim 9 in which determined concentrations of one or more of the following analytes in said fluid sample supports a positive diagnosis of stroke: IL-18 (about 300 pg/mL or above), Factor VII (about 320 ng/mL or above), ICAM-1 (about 170 ng/mL or above), Creatine Kinase-MB (about 5 ng/mL or above), MCP-1 (about 275 pg/mL or above), Myoglobin (about 30 ng/mL or above), C Reactive Protein (about 11 μg/mL or above), TIMP-1 (about 120 ng/mL or above), Ferritin (about 300 ng/mL or above), or Glutathione S-Transferase (about 2 ng/mL or above), or any combination thereof.

Figure 1

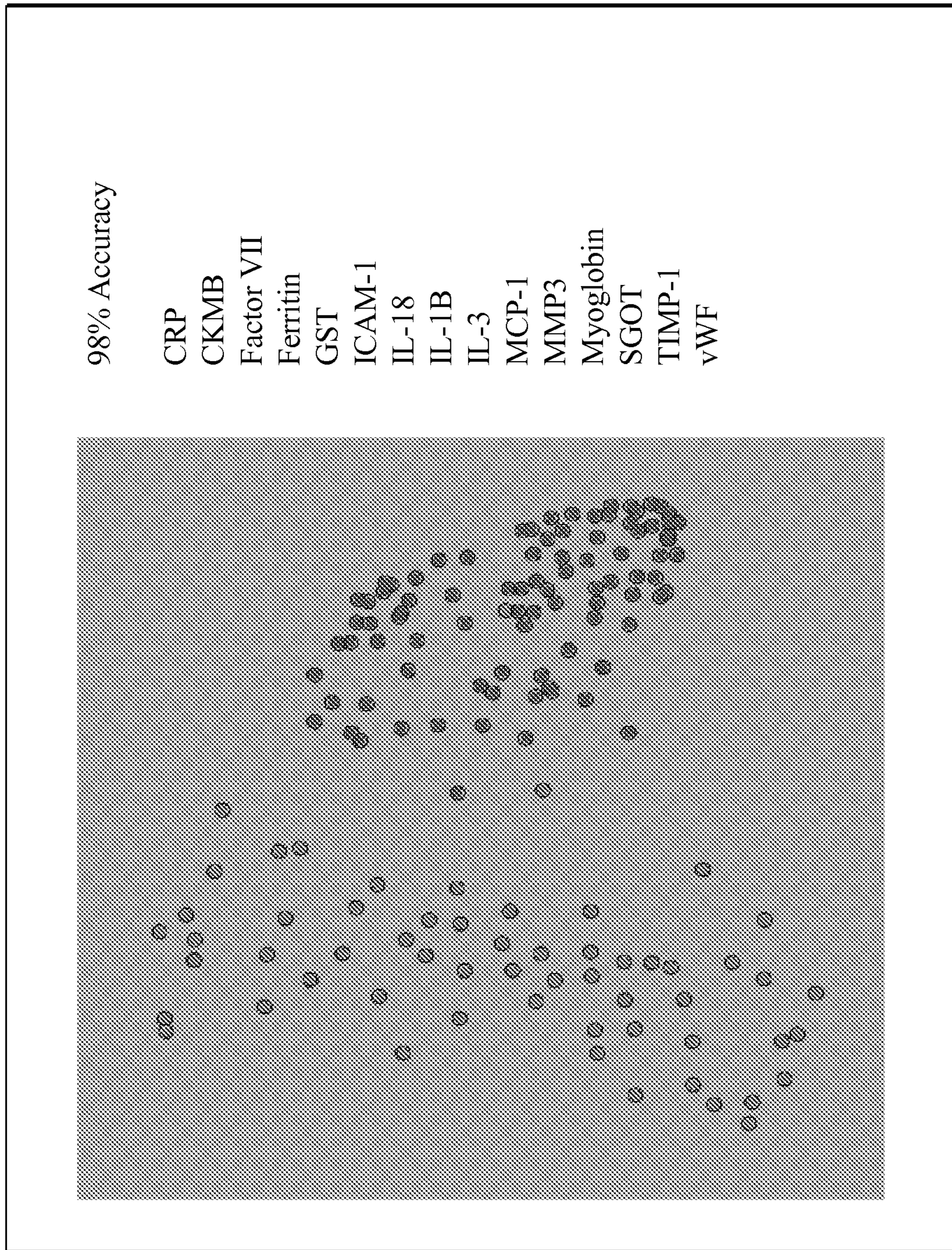
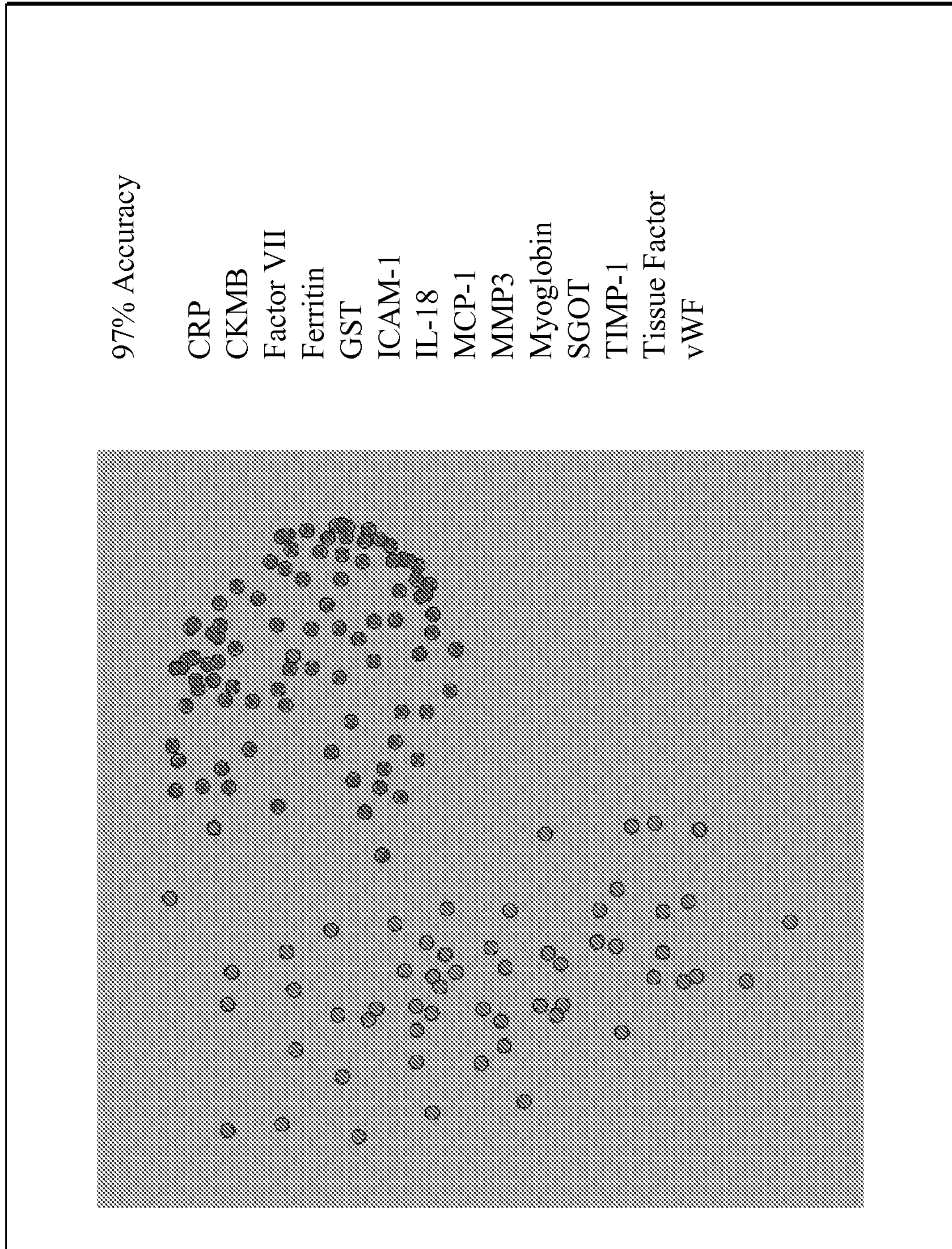


Figure 2



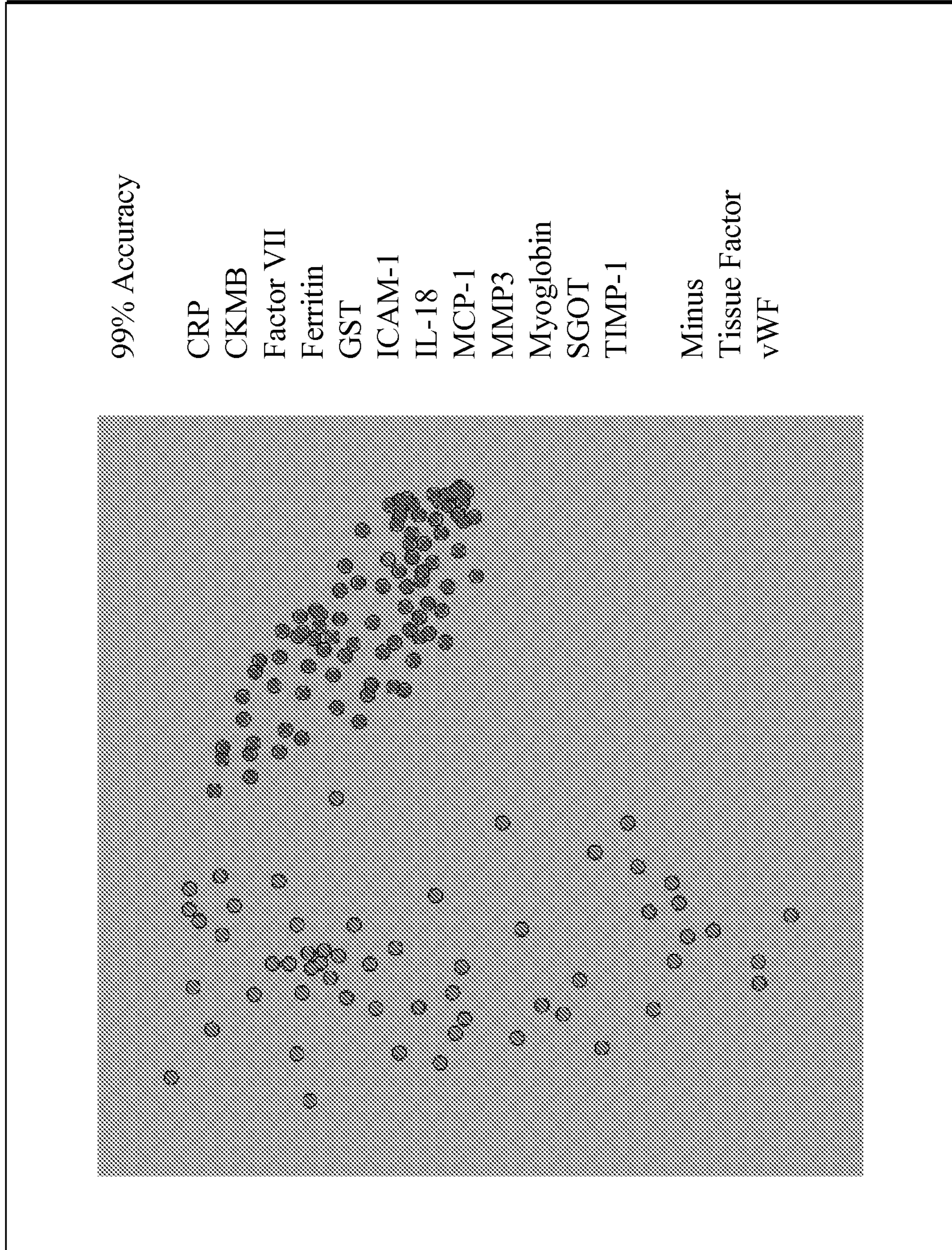


Figure 3

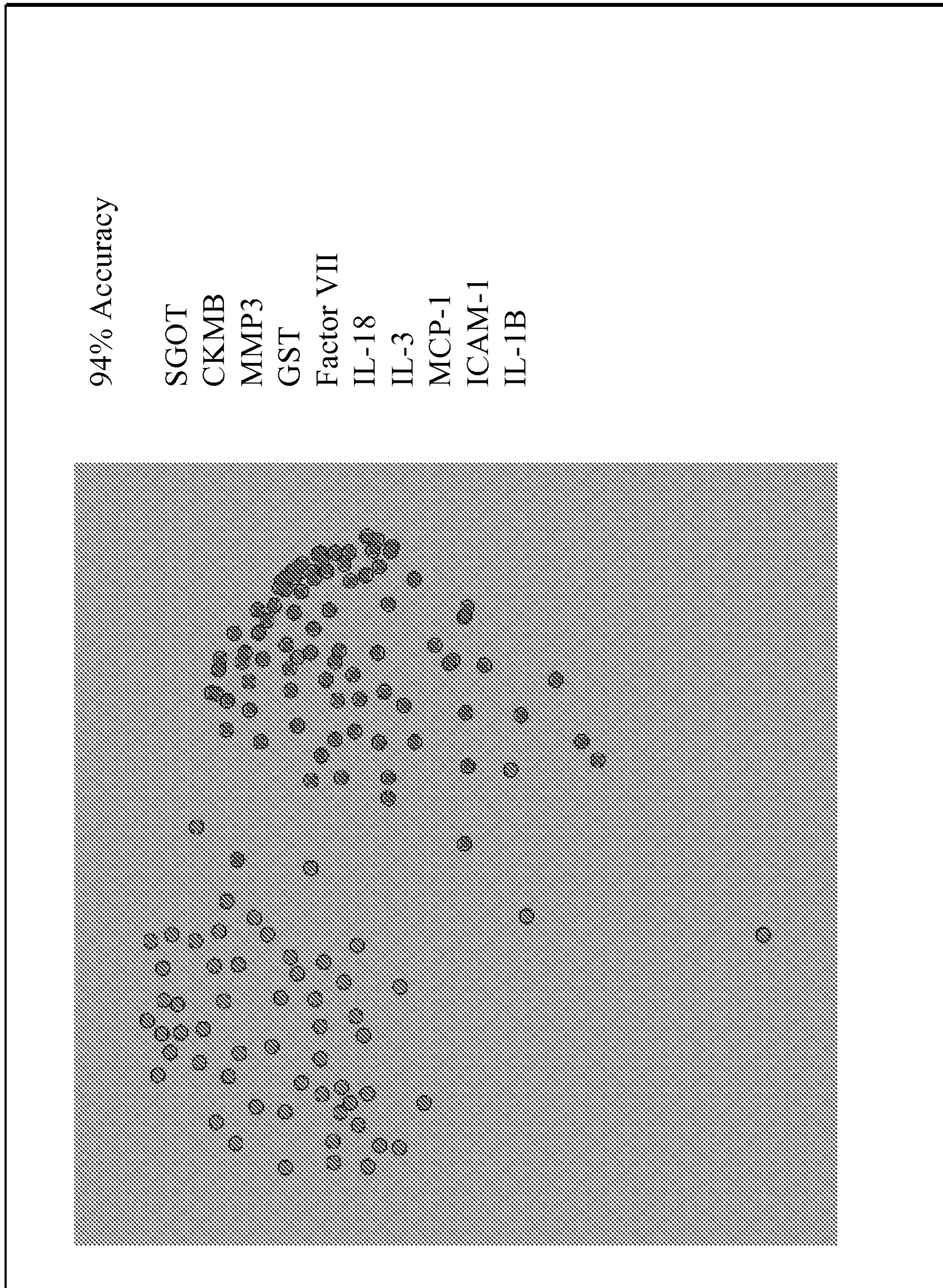


Figure 4

Figure 5