REVIEW ARTICLE
Precision and Future Medicine 2017;1(1):32-42
https://doi.org/10.23838/pfm.2017.00052
pISSN: 2508-7940 · eISSN: 2508-7959
PRECISION AND FUTURE MEDICINE

Advances in biomarker for stroke patients: from marker to regulator

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Received: January 16, 2017
Revised: February 26, 2017
Accepted: February 27, 2017

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ABSTRACT

Biomarkers refer to indicators found in the blood, other body fluids or tissues that predict physiologic or disease states, increased disease risk, or pharmacologic responses to a therapeutic intervention. Stroke is a heterogeneous condition, and stroke biomarkers could be used as a guiding tool for more effective personalized therapy. In this review, the recent advances in the biomarkers in stroke field are discussed. First, various types of biomarkers including genetic, extracellular vesicle, and metabolomics-associated biomarkers as well as protein biomarkers were recently introduced. The studies reviewed herein suggest that comprehensive analysis of different types of stroke biomarkers will improve the understanding of individual pathophysiologies and further promote the development of screening tool of high-risk patients, predicting model of stroke outcome and rational stroke therapy tailored to the characteristics of each case. Second, several biomarkers can be bio-'makers' that regulate compensatory or pathological process in the development of stroke etiology and recovery after stroke. Several protein (e.g., chemokines, caveoli), genetic (e.g., microRNA), and extracellular vesicles (e.g., cancer cell, stem cells-derived) may be directly involved in these processes. These bio-makers may be molecular target of treatment and can be used for new drug development.

Keywords: Biomarkers; Precision medicine; Risk factors; Stroke, ischemic; Therapeutics

INTRODUCTION

Precision medicine relies on biomarkers with which to better classify patients with their disease risk, prognosis, and treatment response. In a narrow sense, biomarkers refer to indicators measured by chemical or biologic tests using blood or urine that predicts physiologic or disease states, or increased disease risk. Biomarkers are also a valuable tool in drug development providing more accurate and complete information regarding drug performance, disease progression, or response to a specific drug therapy. Treatment according to the biomarkers has also been investigated in various diseases including ischemic heart disease, cancer, or immunological disorders. On the contrary, there has been relatively lack of biomarker researches in cerebrovascular disease.

The importance of considering heterogeneity among stroke patients has emerged. Unlike

coronary heart disease, stroke has heterogeneous pathophysiologies and mechanisms. Moreover, individual patients with stroke have different features even among subjects with same stroke mechanisms. These aspects enhance the need for development of personalized medicine based on characteristics of each patient rather than performing large randomized clinical trials.

Herein, we review the current and new stroke biomarkers with their strengths and weaknesses focusing on the importance of comprehensive approaches and the role of biomarkers on the development of new drugs for stroke.

ROLE OF BIOMARKERS IN STROKE RESEARCH

Screening high-risk subjects

Although many attempts including national publicity and various programs for health promotion have been made to manage stroke risk factors, the prevalence of stroke has not been markedly reduced. This may be partially resulted from hidden risk factors of stroke. Interestingly, certain regions in the United States (stroke belt and buckle) have an unusually high incidence and mortality of stroke and the phenomenon could not be explained by the differences of the conventional risk factors [1,2]. Therefore, many researchers have devoted themselves to find nontraditional risk factors of stroke to explain it and numerous possible contributing factors have been identified, including metabolic syndrome, sleep-related breathing disorders, and air pollution [3,4].

In addition to these nontraditional risk factors, a series of biomarkers reflecting inflammation, hemostasis, thrombosis, endothelial function, or neurohormonal activity have been evaluated as potential tools in an effort to improve risk prediction of future stroke, and thereby avert future events [5-12]. Our recent biomarker study showed that although traditional risk factors have been reported to be different between cerebral microangiopathy and macroangiopathy, endothelial dysfunction and related renal dysfunction were associated with both types of cerebral angiopathies [13]. In the near future, genome-wide association study may also greatly contribute to build risk stratification models by identifying genetic variants that confer susceptibility to cerebrovascular disease [14].

Rapid stroke diagnosis

Although the diagnosis of acute stroke mostly relies on neuroimaging techniques, the evaluation of biomarkers of tissue injury would be an alternative strategy for rapid stroke assessment, especially for pre-hospital screening, and fast-tracking in emergency room. A rapid diagnosis of stroke based on biomarkers may be useful especially for pre-hospital screening, facilitating entry into fast track care pathway, and ancillary data when contemplating thrombolysis. However, a widely available, rapid, and sensitive diagnostic test for acute cerebral ischemia has not been available until now.

Recently, a biomarker panel rather than a single marker in isolation has been increasingly used to improve the diagnostic accuracy of suspected stroke. For instance, a diagnostic panel incorporating the levels of matrix metalloproteinase 9 (MMP-9), B-type natriuretic peptide, D-dimer, and S100β into a composite score enhanced sensitivity of early noncontrast computed tomography (CT) alone for acute stroke, although the diagnostic accuracy was clearly imperfect [15]. Furthermore, the approach was feasible as a point-of-care test in the emergency setting [15]. As the number of presumed biomarkers for stroke expands at an exponential rate, it would be expected to develop improved biomarker combinations for more accurate diagnosis of stroke.

Detecting of possible stroke etiology and mechanisms of ischemic injury

Several studies have focused on the use of biomarkers for detecting of possible stroke mechanisms. In addition, the molecular markers related to neuronal death can provide the information about the presence of tissue at risk of infarction [16,17].

Predicting drug response and outcome

It has been well known that different patients respond in different fashions to the same medication. Among many factors that influence the effects of drugs, it is estimated that genetic factor can account for 20% to 95% of variability in drug disposition and effects [18]. For example, previous studies revealed that *CYP2C9* and *VKORC1* genetic variants are associated with warfarin dose requirement and clinical outcomes [19,20].

Besides pharmacogenetics, several biomarkers are also contributing to the predicting drug response in patients with stroke, particularly when thrombolysis is administered. Specifically, elevated S100β and MMP-9 which were reported as serum markers of blood-brain barrier dysfunction before thrombolysis could predict hemorrhagic transformation after thrombolysis [21-23]; whereas, baseline levels of α_2-antiplasmin were predictive of recanalization in patients treated

Table 1. Emerging roles of stroke biomarkers

Role	Example of biomarkers
Screening high-risk (asymptomatic) subjects	CRP, fibrinogen, and inflammatory cytokines, vWF, BNP, and UACR for stroke risk [5-8,10-12] ADMA for silent brain infarcts on MRI [9] Genome-wide association studies of stroke [14]
Rapid stroke diagnosis	Protein biomarkers associated with glial and neuronal cells (S100β, GFAP, NSE, MBP), inflammation (CRP, MMP-9, VCAM, TNF-α, IL-6, VEGF), thrombosis (vWF, D-dimer), and others (BNP, homocysteine) [15,26-28]
Detection of stroke mechanisms and molecular-targeted treatment	Mechanisms of neuronal death, e.g. glutamate, GABA [16,17] Etiologic diagnosis of ischemic stroke, e.g., atherosclerotic (inflammatory markers) vs. cardioembolic (free fatty acid or vWF) vs. cancer-coagulopathy (D-dimer) [29-33]
Predicting drug response	Pharmacogenomics for the resistance to antiplatelet agents, warfarin, statin, or thrombolysis α_2-Antiplasmin for recanalization after thrombolysis [24] MMP-9 and S100β for a high risk of hemorrhagic transformation after thrombolysis [21-23] S100β for delayed infarct expansion [34,35]
Predicting outcome	Inflammatory markers for early neurological worsening [36] and poor outcome after stroke [37] D-dimer for early recurrent ischemic lesion [38] Fibrin-monomer for thrombus formation in the left atrial appendage [39] vWF for adverse events or vascular events in A-fib patients receiving warfarin [40] or aspirin [41] BNP for functional outcome after A-fib stroke [42] BDNF polymorphism for outcome after traumatic brain injury or subarachnoid hemorrhage [43,44] Endothelial EV for predicting future cardiovascular events
Use as surrogate endpoints in clinical trials	Troponin T, CRP, BNP [45] S100β, nitric oxide for phase III trials
Use as new drug development (theranostics)	Chemokines (such as SDF-1α) to improve recovery after stroke [25] Caveolin to improve angiogenesis MicroRNA to improve recovery after stroke Cancer cell-derived EV for coagulopathy and stem cells-derived EV for stroke recovery

CRP, C-reactive protein; vWF, von Willebrand factor; BNP, B-type natriuretic peptide; UACR, urinary albumin/creatinine ratio; ADMA, asymmetrical dimethylarginine; MRI, magnetic resonance imaging; GFAP, glial fibrillary acidic protein; NSE, euron-specific enolase; MBP, myelin basic protein; MMP-9, matrix metalloproteinase-9; VCAM, vascular cell adhesion molecule; TNF-α, tumor necrosis factor-α; IL-6, interleukin 6; VEGF, vascular endothelial growth factor; GABA, γ-aminobutyric acid; A-fib, atrial fibrillation; BDNF, brain-derived neurotrophic factor; EV, extracellular vesicle; SDF-1α, stromal cell-derived factor 1α.

with thrombolysis [24]. There have been accumulating evidences that a number of biomarkers can predict clinical or radiological outcomes from cerebral ischemic events (Table 1) [5-12,14-17,21-45].

TYPE OF BIOMARKERS IN STROKE RESEARCH

Stroke biomarkers include protein, genetic, extracellular vesicle (EV), and metabolomics-associated biomarkers. Each biomarker has different aspects, and its own advantages and drawbacks (Table 2). A recent study reported that multiple EV biomarkers in addition to existing protein biomarkers are valuable for predicting future cardio- and cerebrovascular events [46]. Therefore, comprehensive approach using a variety of biomarkers is warranted to overcome the limitations. In addition, multidisciplinary approaches including neuroimaging biomarkers are needed.

Protein biomarkers

Researches using protein biomarkers in patients with ischemic cerebrovascular disease have mainly focused on pathophysiology, diagnosis, prognostication, and neuronal death in stroke [47]. A typical example of protein biomarkers is C-reactive protein [7,48,49]. However, a recent study raised the possibility that the relation may result from various biases [50]. Moreover, it has become skeptical about efficacy of biomarkers to predict stroke risk because they provide only limited additional information compared to the well-known stroke risk factors [51,52]. Further studies with more systematic approach and analysis are needed in this area.

Genetic biomarkers

Many epidemiological studies suggested that stroke has ge-

Table 2. Strengths and weaknesses of various biomarkers

Type	Advantage	Disadvantage
Protein	Post-stroke analysis Gene-environment interaction Quantitation	Time-dependent and need for serial tests Small numbers of final products (not causative in most)
Gene	Pre-stroke analysis Automatism Plentiful candidate Possible role of pharmacogenomics on drug effectiveness and toxicity	Influence of environment (>gene) on the development of most disease (polygenic) Clinical usefulness of genetic risk factor is low Stroke subtype-specific
MicroRNAs	Both pre- and post-stroke analysis Stably expressed in circulation for several months after stroke onset. Functional and causative	One microRNA has multiple gene targets, and need to examine the combinatorial effect of multiple microRNAs The sample size was usually limited, studies with larger patient cohorts are needed
Extracellular vesicle	Both pre- and post-stroke analysis Functional (it contains nucleic components as well as trophic factors and receptors) May be causative Window for not only damaged but also intact cells and tissues	Methods for measurement are unsettled Effortful Limited and non-specific markers for identification of extracellular vesicles
Metabolomics	Relatively plentiful candidate Monitoring of treatment effects May be functional	Effortful and assay not readily available Difficult to interpret the results (absence of clear cutoff of disease/normal state and intuitive connection between identified species and disease state) Studies in stroke field is relatively lacked

netic susceptibility, and various genetic factors were investigated [53]. However, genome-wide association studies failed to reproduce the positive results obtained from previous studies [54] or the clinical usefulness was very low [55]. For example, the hazard ratio and population attributable risk of hypertension to ischemic stroke is 2.0 and 26%, respectively. Conversely, the genetic influence on stroke was only 1.3 to 1.33 and 11% to 12%, respectively [55]. Genetic risk factors seem to be subtype-sensitive, and differential genetic risk factors have been reported to atherosclerotic, cardioembolic, and lacunar stroke [56]. Further stroke genetic studies might result in better risk prediction for different stroke subtypes and recurrent events, although conflicting result exists [57].

Recently, studies in pharmacogenomic area have been actively carried out. Among them, aspirin, clopidogrel, warfarin, statin, and thrombolytics-related genetic polymorphisms are particularly of interest. It is expected that selecting the type or dose of medication or avoiding side effects or drug resistance may be guided by simple genetic tests in the near future.

Metabolomics

The assumption of metabolomics is that occurrence of the disease is directly related to the specific change of biochemical composition in the cell or biological fluid. Metabolomics-associated biomarker research analyzes profiles of fatty acid, amino acid, or polyamine in the blood or urine and determines normal or pathologic states. Furthermore, metabolomics-associated biomarkers can be applied to the monitoring recovery after treatment. Unfortunately, studies using metabolomics in the area of stroke is relatively lacked.

THERANOSTICS AND BIO-MAKER FOR THE DEVELOPMENT OF NEW DRUGS FOR STROKE

Theranostic nanoparticles that simultaneously deliver both imaging and therapeutic agents have gained significant attention for disease management in recent years. Theranostic agents are able to simultaneously deliver imaging agent and therapeutic drugs. Recent advances in nanomedicine offers new tool for theranostics, several studies using nanoparticles have been applied in stroke animal models. For example, ceria nanoparticles and HSP72-vectorized immunoliposome to protect against ischemic stroke, and fibrin-targeted gold nanoparticles to direct imaging of cerebral thromboemboli

on CT scan [58-60]. However, no theranostic drug have been approved by U.S. Food and Drug Administration for safety concerns.

Innate bioactive biomarker molecules may have pharmacological functions ("bio-maker") and can be used in stroke patients. While nanomedicine-based theranostics provides artificial membrane/compounds, these natural components may role as "safe tools" for theranostics. Therefore, identification and utilization of biomarkers is vitally important for the successful development of disease modifying drugs for diseases. There have been efforts for screening candidates for new treatment strategy among biomarkers to provide a tool for new drug development for stroke. Biochemical products arising from biosynthesis of materials could protect against ischemic injury and recovery after stroke (not from breakdown of brain tissue). Several proteins (e.g., chemokines), genetic (e.g., microRNA), and EVs could be the candidates for bio-maker that regulate compensatory or pathological process in stroke. In addition, several biomarkers (e.g., serum albumin) are related to stroke outcomes and may have a role as natural antioxidants in human. Table 3 shows essential features and examples for natural bio-makers in stroke field.

Natural antioxidants

Albumin is the most prominent protein in serum and a naturally existing antioxidant. In rat model, high-dose albumin has protective effects by reduction of the volumes of brain infarction/swelling and improvement of local perfusion to zones of critical blood flow reduction [61]. Beside albumin, bilirubin, and uric acid have been suggested to have a role as natural antioxidants in the body. Decreased levels of albumin and bilirubin were associated with poor outcome and exogenous application of albumin and uric acid may be beneficial in a certain subgroup of stroke patients (Table 4) [62-70].

MicroRNAs

MicroRNAs are short non-coding RNAs (18–23 nucleotide). MicroRNAs regress target gene expression by mRNA degradation and translation inhibition. Dysregulation of microRNAs has been linked to variety of disease development. Since many

Table 3. Essential features and examples of bio-makers for stroke

Aspect	Essential feature	Example
Roles	Diagnosis (biomarker)+Therapeutics (pharmacologic effects)	Hypoalbuminemia predicts further stroke, and application of albumin reduces infarct size
Source	Naturally exist in human body	Protein (e.g., albumin, chemokines) RNAs (e.g., microRNAs) EVs (e.g., stem cell-derived)
Molecules	Increase by compensatory/rescue mechanisms Decrease by pathophysiologic mechanism	MicroRNA related to recovery/protection Antioxidants and proteins related pathophysiology
Feasibility	Can be regulated/replaced safely with administration	Proteins or EVs

EV, extracellular vesicle.

Table 4. Natural antioxidant in the body

	Albumin	Bilirubin	Uric acid
Blood levels	Related to renal loss of albumin	Reflect intensity of oxidative stress	Decrease with oxidative stress (consumptive?) [62]
Low level	Poor outcome	Poor outcome [63] Increased incidence [64]	Controversial
High level	Not associated	Decreased incidence [65]	Not associated
Clinical trials of exogenous application	Success in a small trial (especially in recanalized patients) [66] but failed in a multicenter RCT (ALIAS trial) [67]	Not done	Failed in a RCT (URICO-ICTUS trial) [68] but improved outcome in selected patients (women or recanalized patients) [69,70]

RCT, randomized clinical trial; ALIAS, albumin treatment for acute ischaemic stroke; URICO-ICTUS, efficacy study of combined treatment with uric acid and r-tPA in acute ischemic stroke.

cell types express a set of microRNAs, microRNA signatures in circulation appear to be tissue-specific, representing their original source. Detectable levels of microRNAs exist in serum with tremendous stability, and circulating microRNAs in the serum can provide a novel class of biomarkers in cancer and other diseases [71]. In stroke field, increased or decreased levels of several circulating microRNA were reported in ischemic stroke patients [72,73]. For example, microRNA-424 level was decreased in acute stroke patients and in an animal model of stroke, and intracerebroventricular injection of lentiviral microRNA-424 reduced ischemic brain injury [72]. On the contrary, post-stroke increase in microRNA-200c contributed to ischemic brain injury by inhibiting reelin expression and inhibition of microRNA-200c resulted in an increase in cell survival [74]. There have been efforts of inhibition and overexpression of microRNAs to attenuate pathologic responses in cardiovascular disease, such as microRNA modulation using antagoMiRs (synthetic reverse compliments of oligonucleotides) that bind and silence target microRNAs or by using microRNA mimicry/pre-microRNAs that perform similarly to endogenous microRNAs [75]. Therefore, microRNAs serve as biomarker for stroke diagnosis and outcomes and also as stroke therapies.

Extracellular vesicles

EVs are defined as a heterogeneous population of small vesicles with a diameter of 0.1 to 1 μm. EVs may be a window for target cell/organs, and include genetic information (i.e., microRNAs) as well as protein inside them [76]. Moreover, it has been identified that EVs have their own function, revealing that EVs from ischemic tissue facilitated vasculogenesis in the ischemic limb model [77]. In this regard, biomarker research using EVs is a prominent field.

Nevertheless, biomarker studies using EVs in stroke are mostly performed in small cohorts. As the methods for analyzing EVs are complicated and not unique mainly due to their very small size, investigations with EVs are currently at a rudimentary state of development. When seeing the results from a large-scale clinical study for prediction of future risk of myocardial infarction, EVs could be a good candidate to compensate limitations of existing biomarker researches [46].

EVs play a critical role in the exchange of information between cells (Fig. 1). For example, stem cell-derived EVs alter the behavior of the target (damaged) cells. In recent studies, EVs secreted from mesenchymal stem cells (MSCs) promoted sciatic nerve regeneration in rats [78]. On the contrary, astrocytes release exosomes enriched in heat shock proteins and synapsin I under stress conditions [79]. Along with others, we

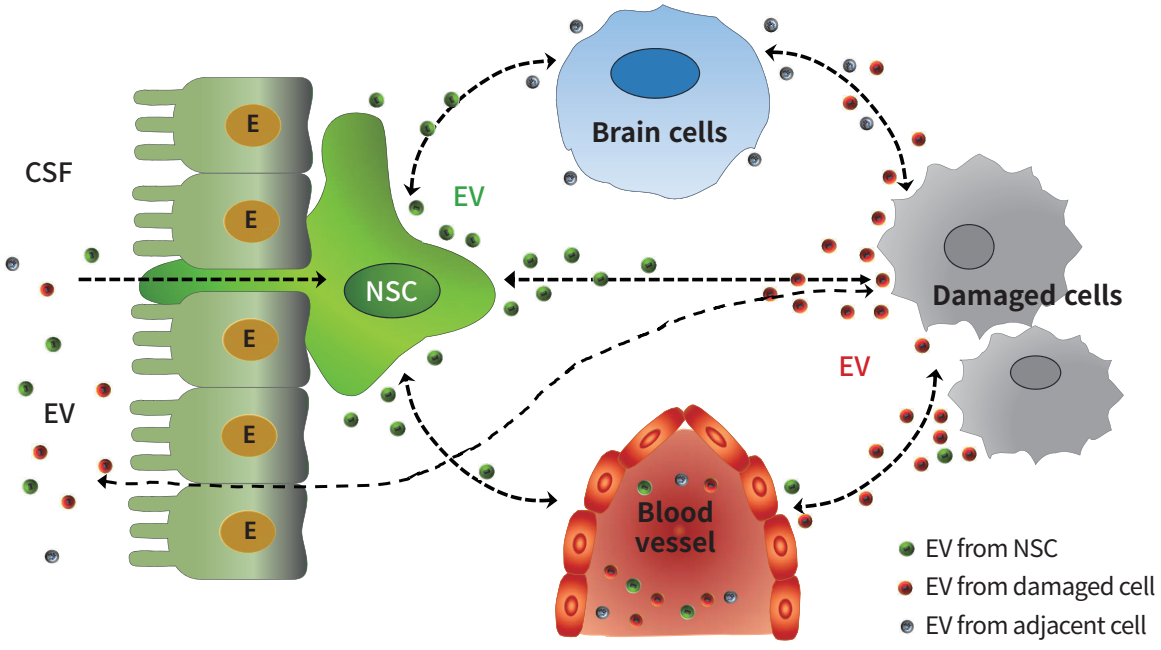

Fig. 1. Diagram outlining inter-cellular communications of extracellular vesicles (EVs) between neurogenic niche and damage cells. EVs released from neural progenitor/stem cells (NSCs) to extracellular milieu promote neuroprotection and neurorestoration after stroke. EVs contain genetic materials, protein and lipid and migration of EVs in the blood vessel and cerebrospinal fluid (CSF) provide the window for tissue in stroke patients.

have shown that intravenous administration of EVs derived from MSC culture media promotes functional recovery and neurovascular plasticity after stroke in rats [80,81]. No studies have examined the effects of stem cell-derived EVs in stroke patients, but a phase 1 study of cord blood-derived MSC EVs in diabetes patients is ongoing (Clinical trial identifier NCT-02138331).

Others

Caveolin

Caveolae are 50- to 100-nm cell surface plasma membrane invaginations that are abundant in endothelial cells and play a major role in the regulation of endothelial vesicular trafficking and signal transduction [82]. Our recent study showed that the serum level of caveolin-1 is decreased in patients with moyamoya disease [83]. Caveolin could be a candidate of a stroke therapy as well as a stroke biomarker. Caveolin-1 is reportedly involved in the pathogenesis of cancers and vascular diseases [82]. Caveolin-1 expression was critical for vascular endothelial growth factor-induced angiogenesis [84] and recruitment of endothelial progenitor cells from the bone marrow [85]. Caveolin expression could be modulated by genetic regulation targeting caveolin-1 using antisense/small interfering RNA or microRNA, anti-caveolin-1 antibodies, and viral vectors or polymer that target the caveolae.

Chemokines

Our previous results have shown that serum level of chemokine (stromal cell-derived factor 1α [SDF-1α]) influences the effects of neurorestorative therapies in stroke patients [86]. The levels of chemokines increase markedly in the infarcted brain during the acute phase of stroke, but decrease over time. Increase the level of SDF-1α in infarcted brain, i.e., target delivery using polymer, could modify the microenvironment to increase innate neurorestorative processes [25].

CONCLUSION

A number of biomarkers are under investigation in patients with ischemic stroke. Currently, however, application of biomarkers is only recommended for research purpose. Monitoring traditional risk factors or vessel status is more efficacious than measuring biomarkers in clinical practice. Considering advantages and disadvantages of each biomarker is important for future study, and comprehensive approach using multiple biomarkers is needed.

The role of biomarker is changing (Fig. 2). It is strongly expected that the biomarkers give us a turning point for investigating pathophysiology and therapeutic mechanisms of ischemic stroke. Continuous efforts are needed to find new biomarker and screening candidates for innate bio-makers

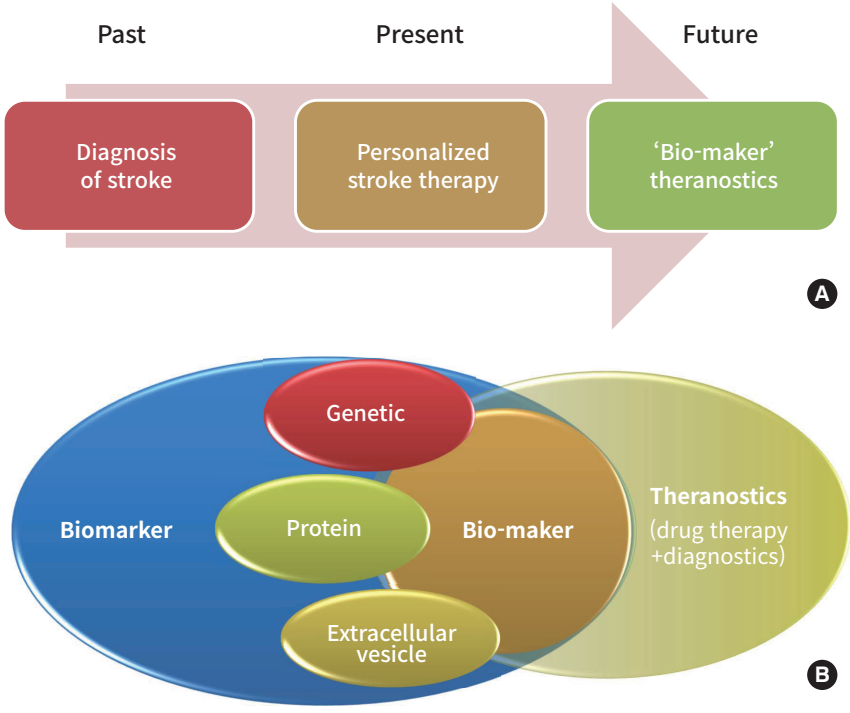

Fig. 2. Overview of biomarkers of stroke. (A) Changes in the role of biomarkers in stroke, (B) biomarker, bio-maker, and theranostics.

among these biomarkers is important for the development of new therapeutic strategy in stroke

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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