Biomarkers for transient ischaemic attack (TIA) and ischaemic stroke

Accurate and early identification of patients with transient ischaemic attack (TIA) to determine those at near-term risk for stroke is imperative and represents an unmet need. Rapid blood tests used in conjunction with neuroimaging for diagnosis of acute ischaemic stroke—including differentiation from haemorrhagic stroke—are essential for initiation of emergency treatment. This article focuses on emerging brain biomarkers used in development of rapid blood assays for diagnoses of TIA and ischaemic stroke. The blood tests may have future potential uses in different clinical settings to improve diagnostic certainty of stroke and benefit patient care.

by Prof. Svetlana A. Dambinova

Stroke is the third most frequent cause of mortality after ischaemic heart disease and cancer, and the first cause of disability in adults worldwide. In 2005, there were an estimated 62 million stroke survivors, with 15 million people suffering a stroke each year. Of these, five million will die as a result, and another five million will be permanently disabled [1]. The most important challenge facing physicians globally is to reduce the unacceptable burden of stroke. Recently, several important issues concerning stroke diagnosis and management, including diagnostic blood testing, were discussed at the 6th World Stroke Congress in Vienna.

The first challenge is to identify the target population in different clinical settings so that a diagnosis of stroke can be determined, such as conducting risk assessment of TIA in persons with pre-existing conditions e.g., hypertension, atherosclerosis, diabetes, cardiovascular disorders— including those that may have occurred subclinically— in primary care offices. It is increasingly understood that TIAs are underrecognised, under-reported and under-treated. After a first TIA, 10% to 20% of patients are likely to have a stroke within the next 90 days; in 50% of these patients, the stroke occurs within the first two days after a TIA [2]. Up to 25% of patients will die within a year of having a TIA [3].

The second challenge is the application of blood tests to the prehospital and emergency management of TIA or stroke. Rapid management of acute ischaemic stroke in the emergency setting, especially if the CT scan is normal or MRI is contraindicated or not available, is essential to ensure that patients receive thrombolysis within the therapeutic window. The ability to speed up ruling in or ruling out of ischaemic stroke prior to CT imaging using a blood test — for example, in the ambulance — would also be extremely useful in this critical diagnostic interval.

The third challenge is the prediction and prevention of adverse neurological events during and after surgery. Patients who experience lower blood pressure during noncardiac surgery may face an increased risk of developing a stroke postsurgery After adjusting for known stroke risk factors such as age, gender, history of diabetes, hypertension and previous stroke, a trend toward a higher risk for stroke after vascular and open heart surgery was found [4,5].

Additionally, educating nonstroke specialists globally on advanced approaches in blood testing for stroke diagnosis will help save lives and decrease disabilities from recurrent strokes. The active role of clinical laboratories in the rapid and accurate assessment of TIA/stroke cannot be underestimated in the hospital and primary care settings. Blood tests classifying patients into low, moderate or high risk disease categories could single out a target group for timely intervention by stroke specialists, to whom patients with highest risk of TIA/stroke should be immediately directed for neuroimaging and administration of novel acute stroke treatments to prevent a major cerebrovascular event.

This article outlines the NMDA receptor peptide and antibody as novel brain biomarkers for TIA and stroke assessment. Based on these biomarkers, two rapid blood assays have been developed and investigated for clinical feasibility in the emergency, in-hospital and surgery settings.

Currently available biomarkers

Stroke is a brain attack that involves multisystemic mechanisms of thrombotic (embolic), neurotoxic coupling that can result in acute and chronic (multiple recurrent TIAs) events. The two principal diagnostic challenges in overall stroke management are difficulties in diagnostic certainty of TIA and differentiating brain ischaemic attacks from haemorrhages and stroke-like disorders. Stroke is a preventable and treatable disease, if it is recognised early, hence the adage: "Time is Brain." However, to date, rapid blood tests for TIA/stroke assessment are still investigational.

Recently, Whiteley et al conducted a systematic review of the literature of brain biomarkers for the diagnosis of ischaemic stroke [6]. They found 21 studies that tested 58 single biomarkers and seven panels of several biomarkers. While high sensitivity or specificity were found for the majority of the biomarkers, limitations to study design and reporting prevented the recommendation of a specific biomarker for use in the clinical setting. Important questions that may be answered by future studies of brain biomarkers are those that address their clinical utility in the management of stroke: whether a patient has had a stroke; whether it is ischaemic or haemorrhagic; and whether symptoms are suggestive of additional intensive investigation or thrombolytic therapy.

During the past five years, a number of molecular and immunochemical assays have been evaluated for clinical use in neurology that detect biomarkers such as S-100B, neuron-specific enolase, glial fibrillary acidic protein (GFAP), brain natriuretic peptide (BNP), D-dimer, matrix metallopeptidase 9 (MMP-9), and monocyte chemotactic protein-1. However, only one assay, the PLAC test (diaDexis, Inc., South San Francisco, CA, USA), which measures the inflammatory marker lipoprotein-associated phospholipase A2 (Lp-PLA2), has been cleared by the FDA for long-term prognosis of risk
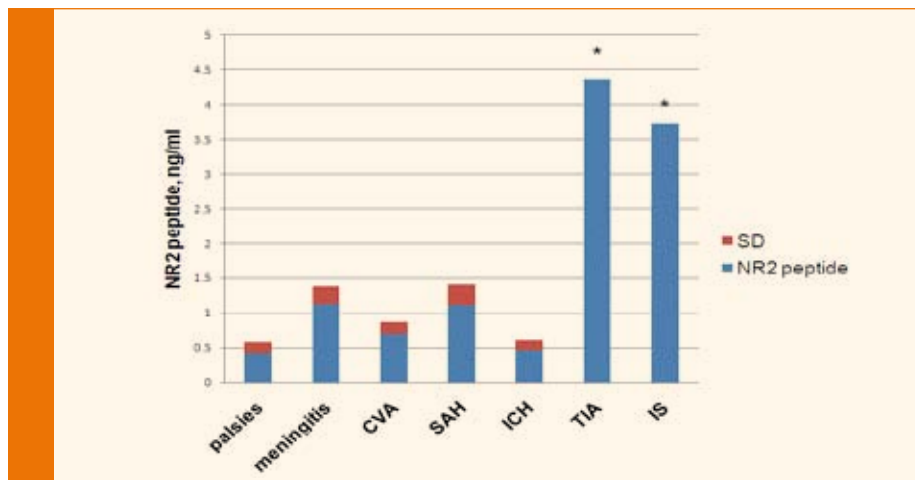
Cardiovascular disease

Figure 1. Distribution of plasma concentrations of NR2 peptide in patients with palsies, meningitis, cerebrovascular accident, subarachnoid haemorrhage, intracerebral haemorrhage, transient ischaemic attack and ischaemic stroke.

for coronary heart disease and stroke (risk ratio 2.03) [7]. A blood assay that can predict near-term risk of TIA and diagnose acute ischaemic stroke would fulfill an enormous unmet need.

Stroke biomarkers in development

The ability to recognise TIA as a temporary (uncompleted) cerebrovascular event requires a brain-borne—not blood-borne—biomarker, which led to work with N-methyl-D-aspartate (NMDA) receptors, major excitatory neuroreceptors that regulate neuronal electrical signals. Substantial progress in understanding the mechanisms of stroke has been made based on findings of the effects of NMDA receptors on cerebral vascular regulation. NMDA receptors are localised on the surface of epithelia of microvessels [8] that form the blood-brain barrier and control microvessel function.

We found NMDA receptor markers to provide real-time evidence of neurotoxicity underlying cerebral ischaemia [9]. An early microembolic process activates NMDA receptor fragment cleavage by thrombin-activated serine proteases [10], resulting in peptide fragments entering the bloodstream [11] through the damaged blood-brain barrier, initiating an immune response and generating antibodies in the blood [12]. On the basis of molecular investigations, NR2 peptide and NR2 antibody were proposed as new markers for stroke and TIA [13], and two tests were developed. The NR2 peptide test detects NMDA receptor peptide fragments, which signal acute ischaemic stroke and TIA (Gold Dot NR2 Peptide test, CIS Biotech, Inc., Atlanta, GA, USA). The NR2 antibody test detects NR2 antibodies to the NMDA

receptor, which can help recognise TIA and prior isolated or multiple cerebrovascular events in patients with pre-existing conditions (Gold Dot NR2 Antibody test, CIS Biotech, Inc., Atlanta, GA, USA). Both tests are CE marked and initiation of clinical trials in the USA is pending U.S. FDA review.

NR2 peptide assay: clinical feasibility

Several studies have been conducted to determine the clinical feasibility of the NR2 peptide assay. A prospective, observational cohort study was performed to assess the ability of the NR2 peptide test to differentiate stroke from stroke mimics in patients with symptoms of acute cerebrovascular accident (CVA) presenting to a community-based emergency department (ED) (Ingham Regional Medical Center, Lansing, MI) [14]. All participants in the study were recruited between October 2004 and December 2005 and consented to treatment. Of the 67 patients (age 56±11 years) enrolled in the study with symptoms suggestive of TIA or CVA, 13 patients had TIA,

19 had CVA as a primary event and 22 suffered a stroke as defined by CT/MRI. The control group included 13 age-matched and gender-matched non-stroke subjects, including 10 individuals with meningitis. Medical records were analysed and results of all pertinent diagnostic tests (including radiologic studies and EEG) were recorded for each patient admitted to the hospital. Acute CVA or TIA was considered present if confirmed either by diagnostic imaging or a combination of neurology consultant impression and final discharge diagnosis. Patients who presented with symptoms considered suggestive of acute stroke (CVA or TIA) by the treating clinician were identified. Analyses of NR2 peptide distribution in patient blood samples are presented in Figure 1. Those diagnosed with a primary CVA event, intracerebral haemorrhage and palsies (stroke mimics) demonstrated low values of NR2 peptide (<0.7 ng/mL), while increased peptide levels were observed in those with meningitis (1.118± 0.263 ng/mL) and subarachnoid haemorrhage (1.105±0.0.304 ng/mL). The NR2 peptide assay significantly discriminated stroke mimics from TIA and ischaemic stroke. The best cut-off value for TIA diagnosis was 1.0 ng/mL (sensitivity, 83%) at which a positive predictive value of 73% was achieved.

A prospective, masked observational study was conducted to determine how this peptide may be affected in acute stroke. Patients presenting with symptoms of acute stroke and a discharge diagnosis of stroke were recruited between January 2006 and January 2007 at DeKalb Medical, Decatur, GA, USA [15]. Of the 89 patients enrolled (45 female, 44 male) with acute or prior ischaemic stroke, 50 patients (26 female, 24 male) with acute stroke, as supported by clinical observations and imaging data, were included in the study. The
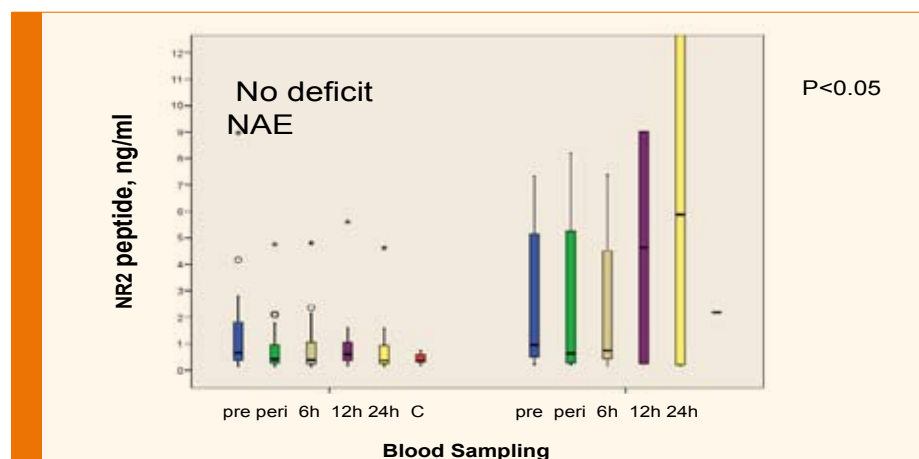

Figure 2. NR2 peptide levels in patients with (n=7) neurological adverse events (NAE) and without deficit for CAS and CEA treatment modalities.
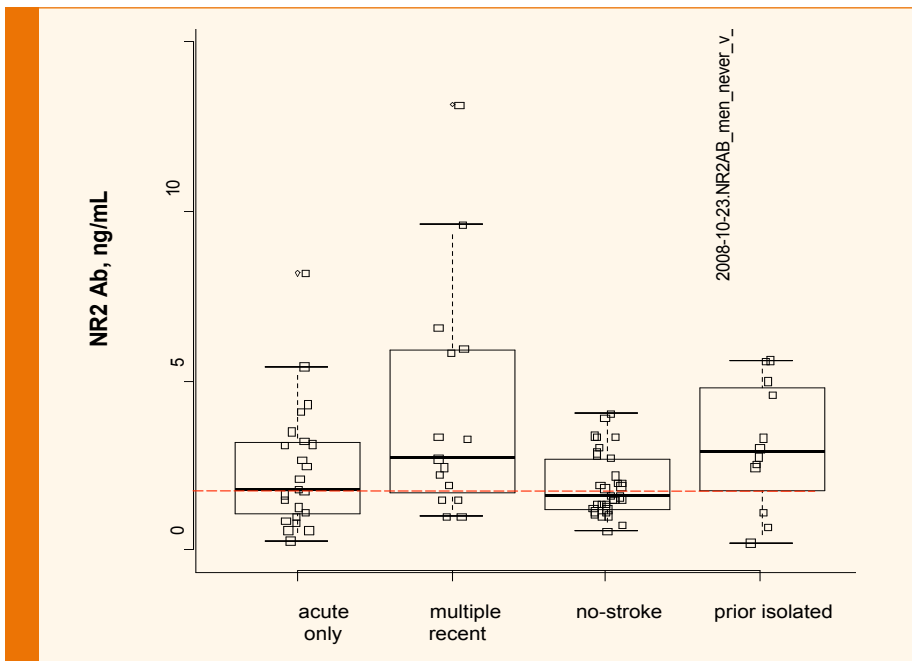
Figure 3. NR2 antibody levels in patients (n=46, men) with acute ischaemic stroke, multiple recent ischaemic stroke, non-stroke, and prior isolated ischaemic stroke. Dotted line shows the cut-off of 2.0 ng/mL.

control group consisted of 30 age-matched and gender-matched non-stroke subjects. Historical and imaging evidence of recurrent stroke were included and compared to NR2 peptide assay results within 72 hours of stroke onset. The primary objective was to assess the ability of the NR2 peptide assay to detect acute stroke. Significant differences in concentrations between the acute stroke (4.91±3.34 ng/mL) and non-stroke control (0.86±0.12 ng/mL) groups were found. The NR2 peptide assay showed 98 % sensitivity in assessing acute ischaemic stroke, with a risk ratio of 16 and a cut-off value of 1.0 ng/mL. Additionally, a significant correlation of NR2 peptide with stroke volume from 2 to 250 cc was observed.

A study was conducted to validate the NR2 peptide as a biomarker for neurological adverse events (NAE) caused by hypoperfusion due to carotid endarterectomy (CEA) and carotid artery stenting (CAS) at the Regional Vascular Unit, St Mary's Hospital, London, UK [16]. Data were collected prospectively from 51 patients and divided into two groups of 27 and 24, representing patients undergoing CEA and CAS, respectively. Magnetic resonance angiography (MRA) of the carotid arteries, along with the intra-cerebral vessels (including the circle of Willis), was conducted for each patient. All patients underwent CT scans before CAS and CEA to evaluate carotid plaque and its stability, and CT perfusion scanning was used to assess brain blood flow changes. NR2-peptide concentrations

for all NAE cases (n=7) were significantly increased compared to patients without neurological deficit [n=37, Figure 2]. Clinical events were correlated with NR2 peptide levels in NAE patients before, during, and after both procedures [Figure 2]. Furthermore, the NR2 peptide levels increased significantly in those patients with NAE in the postoperative period for either procedure (p=0.006 for CAS and p=0.005 for CEA) compared with their preoperative biomarker levels. The high diagnostic power for the NR2-peptide assay in assessing NAE with a sensitivity of 99% was calculated.

NR2 antibody assay clinical validation

Patients presenting with symptoms of recent TIA, acute stroke and a discharge diagnosis of stroke defined by neuroimaging and registered in histories at DeKalb Medical, Decatur, GA, USA were recruited between January 2006 and January 2007. Of the 46 male patients with cerebrovascular events, 44 were determined to have acute or prior ischaemic stroke, two had TIA and one had intracerebral haemorrhage (ICH) [17]. The control group consisted of age/gender matched 24 subjects without a history of stroke, including seven individuals with controlled hypertension and two with diabetes mellitus. NR2 antibody levels were higher in the multiple recent group compared to acute and non-stroke groups [Figure 3]. Patients with prior isolated stroke had significantly higher NR2 antibody concentrations com-

pared to non-stroke persons. The detailed analysis of five different cutoff values for NR2 antibody concentrations detected in stroke patients with lesion volumes within 5-70 cc yielded the best test sensitivity (95.9%) at a cutoff value of 2.0 ng/mL with the near term risk ratio for stroke of 3.45. The analysis of the NR2 antibody test performance for TIA assessment had the highest risk ratio of 32.9 at a cut-off value of 2.0 ng/mL with test sensitivity of 98%.

To determine the efficacy of the NR2 antibody test as a predictor of risk of TIA/stroke, NR2 antibody levels were measured in 1129 banked serum samples from a retrospective, masked, multicentre observational study involving 33 centres in the United States [4]. Inclusion criteria included high-risk adult patients (aged >18 years) undergoing cardiac surgery with cardiopulmonary bypass, such as combined coronary artery bypass graft (CABG) and valve surgery, or urgent and emergent CABG or valve surgery. Samples used for the NR2 antibody test were from participants aged 48-80 years. This was a case-cohort study, where samples from all the cases (373) were tested together with 211 appropriately matched healthy participants (controls). A total of 96% (24/25) of patients with NR2 antibodies concentrations ≥2.0 ng/mL preoperatively had neurological complications within 48 hours post-CPB vs only 5.4% of patients with NR2 antibodies concentrations ≤2.0 ng/mL, resulting in a 17.9-fold increase (95% CI, 11.6–27.6) in the predictive ability of a postoperative adverse neurological event. At least 30% of patients who underwent cardiac surgery had neurocognitive deficit postoperatively. Based on the obtained likelihood ratio of a neuroevent of 17.9, an NR2 antibody concentration ≥2.0 ng/mL detected preoperatively will predict neurological complications in 89% of patients after surgery.

Conclusions

Rapid diagnosis of both TIA and stroke is essential for optimal patient treatment and outcome. Clinical use of blood tests that can reliably detect or exclude a cerebrovascular event, predict consequences, or forecast recovery and outcome, might improve diagnostic certainty of TIA/stroke.

Early identification of TIA based on a blood assay that can detect brain-borne biomarkers, such as the NR2 peptide and NR2 antibody, has the potential to become a key component of a successful treatment strategy and outcome monitoring.

Advances in analytical assay technologies have made it possible to develop a rapid, cost-effective brain panel that can be used to predict TIA in a target population (e.g., those with pre-existing conditions) and to select a high risk group for the immediate attention of a stroke specialist.

Timely assessment of TIA/stroke includes educating nonstroke specialists (primary care physicians, cardiologists, vascular surgeons, anesthesiologists, and diabetologists) in new approaches to early diagnosis with the use of appropriate blood tests. Rapid results of blood tests have the potential to shorten the time patients suspected of having a TIA or stroke are seen by neurologists. This can benefit the healthcare system, not only by improving stroke management but by saving lives and reducing costs resulting from disabilities worldwide.

References

1. Mackay J, Mensah G. The Atlas of Heart Disease and Stroke. World Health Organisation 2004; 50-51.

2. GW Albers, Caplan LR, Easton JD, Fayad PB, Mohr JP, Saver JL, Sherman DG. Transient ischemic attack—proposal for a new definition. N Engl J Med 2002; 347(21): 1713-1716.

3. Hill MD, Yiannakoulias N, Jeerakathil T, Tu JV, Svenson LW, Schopflocher DP. The high risk of stroke immediately after transient ischemic attack: a population based study. Neurology 2004; 62: 2015-2020.

4. Bokesch PM, Izykenova GA, Justice JB, Easley KA, Dambinova SA. NMDA receptor antibodies predict adverse neurological outcome after cardiac surgery in high-risk patients. Stroke 2006; 37: 1432-1436.

5. Brightwell RE, Sherwood RA, Athanasiou T, Hamady M, Cheshire NJW. The neurological morbidity of carotid revascularisation: using markers of cellular brain injury to compare CEA and CAS. Eur J Vasc Endovasc Surg 2007; 34: 552-560.

6. Whiteley W, Tseng MC, Sandercock P. Blood biomarkers in the diagnosis of ischemic stroke. A systematic review. Stroke 2008; 39: 2902-2909.

7. Gorelick PB. Lipoprotein-associated phospholipase A2 and risk of stroke. Am J Cardiol 2008; 101(12A): 34F-40F.

8. Sharp CD, Fowler M, Jackson TH, Houghton J, Warren A, Nanda A. Human neuroepithelial cells express NMDA receptors. BMC Neurosci 2003; 4: 28.

9. Dambinova S, Izykenova G, Gappoeva M, Wang Y, Hoffer B. NMDA receptors expression and immunoreactivity in experimental cerebral ischemia and hemorrhage. J Neurochem 2003; 87: S144.

10. Gingrich MD, Traynelis SF. Serine proteases and brain damage—is there a link? Trends Neurosci 2000; 23: 399-407.

11. Dambinova SA, Khounteev GA, Izykenova GA, Zavolokov IG, Ilyukhina AY, Skoromets AA. Blood test detecting autoantibodies to N-methyl-d-aspartate neuroreceptors for evaluation of patients with transient ischemic attack and stroke. Clin Chem 2003; 49: 1752-1762.

12. Dambinova SA, Khounteev GA, Skoromets AA. Multiple panel of markers for TIA/stroke evaluation [Letter]. Stroke 2002; 33: 1181-1182.

13. Dambinova SA. Brain Biomarkers for Cerebral Ischemia: NMDA Receptor Degradation and Blood Assay Development. AACC Press. Forthcoming.

14. Glynn T, Tews M, Izykenova G, Hughes M, Khunteev G, Dambinova S. The clinical utility of serum NR2 peptide assay in the diagnosis of patients presenting to the emergency department with acute cerebrovascular ischemic events. Ann Emerg Med 2007; 50: S35. Abstract 107.

15. Weissman JD, Khunteev GA, Olson D, Izykenova G, Dambinova S. NR2 peptide levels correlate with acute cortical stroke volume and may detect reversible ischemia. Stroke 2007; 38: 494. Abstract P39.

16. Dambinova SA, Brightwell RE, Khunteev GA, Cheshire NJ. NR2 peptide correlates with acute cerebrovascular events after carotid revascularization. Submitted to Stroke.

17. Dambinova Sa, Khunteev GA, Olson DA, Izykenova GA, Heath R, Fedanov AV, Weissman JD. NR2 peptide indicates transient ischemic attack and acute ischemic stroke. Int J Stroke 2008; 3: 153-154. Abstract PO01-246.

The author

Svetlana A. Dambinova, DSc, PhD
Visiting Professor
Emory University • Atlanta, GA, USA
Tel +1 404 325 9343
e-mail: sdambin@emory.edu
Prof. Dambinova is a consultant to CIS Biotech, Inc. (Atlanta, GA, USA), GRACE Laboratories, LLC (Atlanta, GA, USA), and Siemens Medical Diagnostics Solutions (Tarrytown, NY, USA).
i www.cli-online.com

Biomarkers for TIA and ischaemic stroke

Pg.7

Also in this issue :
EPCA-2 in PCa Pg. 20 | Flow cytometry for rare-event analysis Pg. 24 | Iron deficiency anaemia Pg. 34