Blood Biologic Markers of Stroke: Improved Management, Reduced Cost?

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Identifying blood biomarkers may be of particular value in neurologic disorders such as stroke because of the difficulty in directly studying the brain and its blood vessels. Markers of brain injury, inflammation, excitotoxicity, and oxidative damage have been evaluated for their value in stroke diagnosis, treatment, and management, but none has proved to be sensitive or specific enough for routine clinical use. However, new cellular and molecular profiling approaches using the peripheral blood offer the potential for identifying panels of genes and proteins by increasing specificity while maintaining sensitivity. Furthermore, the first biomarker for predicting stroke risk associated with atherosclerosis (lipoprotein-associated phospholipase A_2) was recently approved by the United States Food and Drug Administration. The ultimate aim for stroke biomarkers is to develop rapid, easy to use, widely available, and inexpensive diagnostic tests that can be used in the clinic and in clinical trials.

Introduction

Stroke is a leading consequence of atherosclerotic vascular disease and is the third leading cause of death and the leading cause of adult disability in the United States and developed countries, and consequently impacts considerably on health care costs. The current stroke management paradigm relies heavily on clinical diagnosis [1]. The typical sequence of steps in managing a stroke patient is to 1) confirm the diagnosis of stroke; 2) determine what type of stroke it is (ischemic or hemorrhagic); 3) determine if treatment with recombinant tissue plasminogen activator (rt-PA) therapy is appropriate; 4) determine the risk of bleeding after rt-PA therapy; 5) determine the likely prognosis; 6) determine the stroke mechanism (eg, embolic); and 7) determine the risk of stroke recurrence. However, the answers to some of these questions are imperfect at best [2]. Only about 70% to 80% of patients with an initial suspected diagnosis of stroke turn out to have a stroke [3,4].

The use of additional laboratory markers of these processes would be most welcome, especially if they could be proven to be accurate, rapid, and easily performed in clinical practice. This is particularly so in neurologic disorders such as stroke because of the inability to directly study the brain and its blood vessels. Biopsy is rarely available or acceptable. Neuroimaging techniques such as CT and MRI, magnetic resonance angiography, and ultrasound have proven to be invaluable for stroke diagnosis and for utility in prognosis and stroke risk. CT is particularly reliable for the diagnosis of acute intracerebral hemorrhage [5] and diffusion-weighted MRI for the diagnosis and prognosis of ischemic stroke [6], but these are time consuming and costly to perform and have limited availability.

With the advent of rt-PA and a number of promising new treatments on the horizon (eg, factor VIIa for intracerebral hemorrhage [7] and the neuroprotective agent NXY-059 [8]) that need to be administered within the first 3 to 6 hours of stroke, interest is returning to the use of the blood to find a rapid, widely available test for the early diagnosis of stroke. Blood tests have the potential to reduce the use of costly procedures and to be used in the home or ambulance setting. The blood is the most practical source of tissue in the clinical setting; it may reflect systemic changes to disease and may permit the development of rapid diagnostic tests along with the evaluation of the pharmacodynamic properties of novel therapeutic agents and responses to therapy. The search for blood biomarkers of stroke and stroke risk has been going on for more than four decades [9], but so far only one marker has been approved by the US Food and Drug Administration (FDA). During this time here has been a dramatic improvement in the understanding of the pathophysiology and molecular and biochemical mechanisms underlying acute stroke and atherosclerotic vascular disease, along with improved methodologies for profiling of the peripheral blood. These advances are providing new avenues for finding blood-based biomarkers of stroke and stroke risk from all of the blood elements,

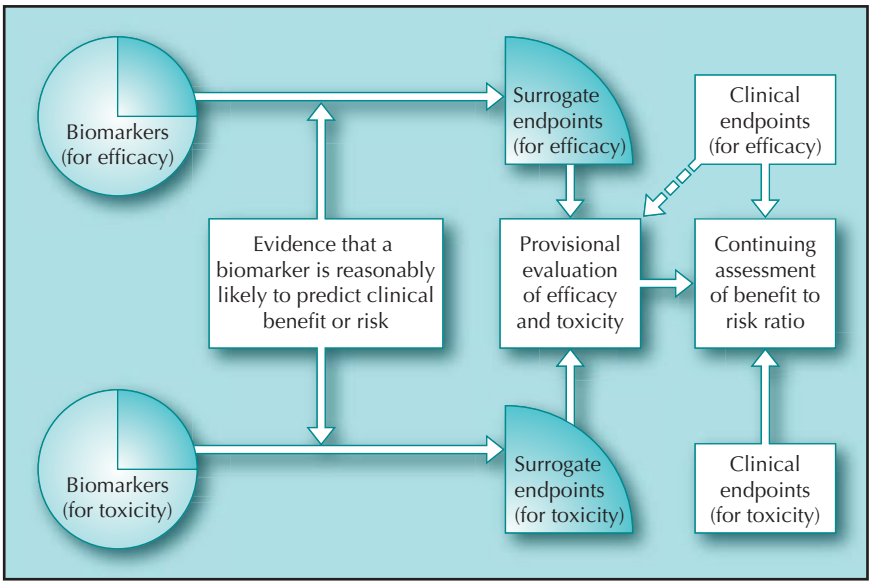

Figure 1. Conceptual model of the relationship of biomarkers, surrogate endpoints, and the process of evaluating therapeutic intervention. (*Adapted from* Biomarkers Definitions Working Group [10].)

including genomic and flow cytometric profiling of the peripheral leukocytes and endothelial cells, and the entire proteome in the plasma and the serum.

In this review, the history and changing approaches to the development of biomarkers are addressed, especially focusing on emerging trends in blood biomarker studies for stroke and for stroke risk.

Biomarkers, Surrogate Markers, and Issues of Disease Association and Causality

In 2001, the Biomarkers Definitions Working Group [10] developed standards and definitions for biomarkers in recognition of rapid advances in molecular biology and the sequencing of the human genome, the growing interest in the use of biomarkers as clinical and/or surrogate endpoints in clinical trials, and because of the increasing number of potential molecular therapeutic targets with the need for rapid evaluation. The following definitions were developed [10]:

- Biologic marker (biomarker): "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention." Biomarkers are developed from a wide array of analytical tools to measure biologic parameters.

- Clinical endpoint: "used in clinical trials and is a characteristic or variable that reflects how a patient feels, functions, or survives."

- Surrogate marker: "a biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit (or harm or lack of benefit or harm) based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence."

Biomarkers have potential clinical utility in a number of clinical areas [10,11]. Examples of commonly used biomarkers are those for the diagnosis of acute myocardial infarction (creatine kinase MB isoenzyme and troponin) and B-type natriuretic peptide for heart failure. There is also an important role of biomarkers in clinical trials for determining dosing and identifying factors determining therapeutic response. Surrogate markers are particularly valuable for improving the efficiency of clinical trials, by reducing the time taken to conduct the trial, particularly in phases I and II. The major limitation of biomarkers is that they can never substitute for a clinical measurement such as a clinical endpoint in a clinical trial, a striking example being that of ventricular arrhythmias in the Cardiac Arrhythmia Suppression Trial [12], in which an improvement in the surrogate endpoint was seen in the treatment arm while in fact the mortality was higher. Examples of surrogate endpoints that have been in long-standing use are blood pressure and blood cholesterol levels, which are used in clinical trials of antihypertensive agents and lipid-lowering drugs. A conceptual model showing the potential uses of biomarkers in clinical trials was developed by the Biomarkers Definitions Working Group and is shown in Figure 1 [10]. In Figure 2, a model showing the potential effects of therapeutic interventions on biomarkers and clinical endpoints in clinical trials is shown, demonstrating how a biomarker must capture sufficient beneficial and harmful effects to be acceptable as a surrogate endpoint [10].

The features of an ideal biomarker are shown in Table 1 [10,11]. Five key features are 1) that a biomarker adds independent clinical information (eg, about risk or prognosis); 2) that it should account for a large proportion of the risk associated with a given disease or condition; 3) that it should be reproducible; 4) that if it is to be used

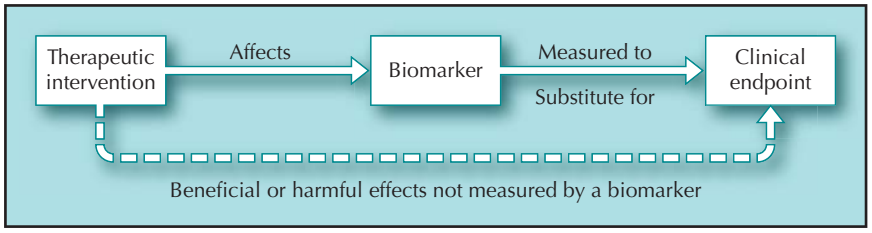

Figure 2. Effects of therapeutic intervention on biomarkers and clinical endpoints in clinical trials. In many circumstances, a therapeutic intervention will affect a clinical endpoint in a way that is not entirely accounted for by its effect on a biomarker. This is likely to occur in complex diseases in which a single biomarker may capture only a portion, or none, of the treatment effect. Interventions may also have unanticipated adverse consequences that diminish or completely offset the intended therapeutic benefits. The independent impact of these unanticipated beneficial or harmful effects of an intervention on clinical endpoints is represented by the *broken arrow*. Those biomarkers that do not account for a sufficient proportion of the treatment effect do not advance to surrogate endpoint status. (*Adapted from* Biomarkers Definitions Working Group [10].)

as a diagnostic test, it should be sensitive and specific and have a high predictive value; and 5) that the test should be readily available [13]. Cost effectiveness is also an important consideration.

Novel biomarkers provide substantial opportunities to improve risk prediction but several important issues relate to the evaluation of their clinical utility. Firstly, the finding of an association or correlation is not enough. Even finding independence of the biomarker from other clinical parameters may not even be enough [14]. For true clinical or scientific utility, a biomarker needs to be shown to be causal, or to add substantial additional power to existing clinical tools and predictive algorithms, and/or to be of value as a surrogate marker in clinical trials. One way of showing that the biomarker adds substantially to existing paradigms, for example, is by the use of a receiver operating curve analysis [14,15], in which the additional percentage increase in accuracy provided by a new biomarker can be determined. Secondly, the finding of association does not imply causality: examples of ways by which causality may be evaluated are to 1) see if the biomarker is specific for a disease state; 2) see if functional genetic polymorphisms influence the disease; and 3) see if giving treatment that has been shown to affect the disease reduces the level of the biomarker. Other considerations are whether the association of a biomarker with disease preceded the clinical event (ie, is a risk factor) or was a consequence of it, an issue with current inflammatory biomarkers (eg, elevated leukocyte counts in patients with acute ischemic stroke [16,17]). Ways of distinguishing between whether the marker is a risk factor or not for disease are by conducting intervention and prospective epidemiologic studies. Established biomarkers should ideally also be proven to be cost effective.

Stroke and Biomarkers

Acute stroke and single blood markers

The search for blood diagnostic markers of acute stroke has been going on for about 50 years. In early studies, lactic dehydrogenase and aspartate aminotransferase were investigated [9]. By 1967, the potential role of creatine kinase as a marker for stroke was being tested [18]. By the mid 1970s and 1980s, it was possible to examine individual nervous system–based proteins and their fragments, including the BB isoenzyme of creatine kinase (CK BB) [19–21]. CK-BB is predominantly found in brain tissue but is not normally present in measurable amounts in the serum of normal adults. The BB isoenzyme fragment of creatine kinase was detectable in trace amounts after ischemic stroke in several studies but had a short half-life and needed very sensitive assays to be detected [19–21]. In other studies, substances released from degenerating neurons and glial cells were evaluated as potential diagnostics for stroke, including neuron-specific enolase, S100β (a marker of glial activation), and glial fibrillary acid protein (released from astrocytes), but these were not proven to be sensitive or specific enough for clinical use [22,23].

Over the past two to three decades there has been a dramatic improvement in the understanding of the molecular mechanisms and pathophysiologic processes underlying stroke [24–26]. The ischemic focus has been found to consist of a central zone of severely reduced blood flow (ischemic core) surrounded by a zone of mild to moderate reduction in blood flow (ischemic penumbra) that is nourished by collateral blood vessels [24–26]. Different biochemical and molecular thresholds of injury and viability have been found at different levels of blood flow reduction and, therefore, in the ischemic core and the penumbra, opening up multiple additional avenues for finding biomarkers of stroke and stroke risk, particularly of excitotoxicity and inflammation. Examples of potential plasma and/or serum biomarkers that have been used to investigate excitotoxicity are glutamate, gamma aminobutyric acid (GABA), and glycine. Examples of potential biomarkers to investigate inflammation are high sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1), and matrix metalloproteinase-

Table 1. Ideal properties of biologic markers

Properties and uses	Ideal properties
Physical properties	Stable, not susceptible to generation of artefacts or loss during processing and storage
	Independence of diet or dysmetabolism
Analytical properties	Extremely sensitive
	Specific
	Reproducible
Clinical and scientific validity properties	Biomarker levels reflect different degrees of brain injury Biomarker levels reflect different stages of clinical severity
	Changes in biomarker levels correspond closely to changes in patient's clinical status or prognosis
	Changes in biomarker levels induced by therapeutic intervention translate into clinical improvement
	Lack of influence of disease symptoms or signs on the marker
Practical properties	Minimal invasiveness or patient discomfort ("subject friendly")
	Low per-usage cost (this is especially important if widespread screening is contemplated)
	Wide availability at treatment centers in all desired geographic locations
Uses	Drug development, favoring early evaluation of efficacy and safety of new drugs
	Potential tool for predicting individual response to treatment
	Contribute to the understanding of the molecular and cellular mechanisms responsible for the clinical response
	If the biomarker level is influenced by the drug dose, its use in preclinical trials might improve the dose range definition

(*Adapted* from Biomarkers Definitions Working Group [10].)

9 (MMP-9) [27]. Examples of potential biomarkers of coagulation are fibrinogen, vascular cell adhesion molecule (VCAM), plasminogen activator inhibitor-1 (PAI-1), and von Willebrand factor. Examples of potential biomarkers used to investigate oxidative stress are ascorbic acid, alpha-tocopherol, uric acid, and superoxide dismutase [28]. Other potential processes that could be investigated are markers of the ischemic core, markers of the ischemic penumbra, markers of reperfusion injury, and markers of blood-brain barrier disruption.

In recent years, studies have focused on the use of inflammatory and excitotoxic biomarkers for their value in identifying patients at risk of neurologic worsening or of hemorrhage after rt-PA therapy (representative examples are shown in Table 2). For example, elevated levels of proinflammatory cytokines have been associated with neurologic worsening [29], along with elevated levels of glutamate. Elevations in MMP-9 have been associated with an increased risk of hemorrhagic transformation after rt-PA [30] along with cellular fibronectin. Most of these potential biomarkers were measured in the serum or plasma and most were studied in isolation. But despite the numerous reports of correlations, none of these have gone into practice. There are many reasons for this. First, in the case of correlations with neurologic worsening, multiple markers (eg, glutamate, Il-6, and total leukocyte count) were shown to correlate with outcome (the initial basis for these studies being to evaluate the scientific correlations of excitotoxic and inflammatory processes with stroke in human patients). However, it is not clear which marker is of the most use clinically. Interactions among these various markers could be performed using a biologic modeling approach, but this has not yet been done. Other biomarkers have either not been tested fully for their potential clinical utility (in terms of demonstrating high accuracy and precision and clinical reproducibilty) or otherwise (eg, in the case of oxidative markers [28]) the assays are not robust enough for clinical use. It has also been suggested that the lack of available clinical assays has hampered progress in the field [27]. Another factor could be lack of reproducibility of some clinical studies, although MMP-9 is showing up in a number of studies involving gene and protein panels (see following text) and so may go on to be a useful test. In some studies, potential biomarkers have been shown to give independent prognostic information in addition to clinical factors (eg, total leukocyte count) but these have not gone on to be applied in clinical practice, perhaps because of issues of specificity or because clinical algorithms have not been developed.

Table 2. Representative examples of potential blood biomarkers being studied in stroke

Clinical application	Examples studied
Ischemic stroke diagnosis	Creatine kinase BB isoenzyme [19–21]
	S100β [22,23]
	Neuron specific enolase [22,23]
	Panel of 22 genes [34••]
	Panel of 4 proteins [36••]
	Panel of 5 proteins (ongoing BRAIN study)
	PARK7, nucleoside diphosphate kinase A [38]
Stroke type	
Ischemic versus hemorrhagic	ApoC-I and ApoC-III [39•]
Bleeding risk after rt-PA	Matrix metalloproteinase-9 [30]
Prognosis	
Early deterioration	Glutamate [27]
	Glycine [27]
	Reduced levels of gamma aminobutyric acid [27]
	Nitric oxide [27]
	Interleukin-6 [29]
Malignant MCA syndrome	Matrix metalloproteinase-9 [27]
Stroke recurrence and death	CD4+CD28- T cells [42]
Stroke risk	Lp-PLA_2 [47••]
	hsCRP [48•,50]
	Total leukocyte count [16,17]

ApoC—apolipoprotein C; BRAIN—Biomarker Rapid Assessment of Ischemic iNjury; hsCRP—high-sensitivity C-reactive protein; Lp-PLA_2—lipoprotein-associated phospholipase A_2; MCA—middle cerebral artery; rt-PA—recombinant tissue plasminogen activator.

Novel approaches and molecular and cellular profiling approaches

The newest approach to finding biomarkers is to look for panels of genes or proteins in the blood in an effort to increase specificity while maintaining sensitivity. These involve the "discovery" approaches of genomics and proteomics [31]. Advances in technology and in the understanding and sequencing of the human genome permit hundreds and thousands of genes and proteins to be screened at one sitting using small chips and elaborate scanning machines. Therefore, it is hoped that groups or panels of genes or proteins that are disease specific are more likely to be detected and be more rapidly detected with these approaches. The potential drawbacks are the overwhelming amount of data that is generated, the complexity and the cost of these methods, and the need for bioinformatic expertise. As these features may not be found in a single biomarker, an alternative strategy might involve combining several different biomarkers with distinct properties to gain both sensitivity and specificity. However, as in the case of single biomarkers, the same scientific and clinical issues alluded to previously will apply. Systems biology approaches have recently been used to look at networks and pathways among genes and could be used to study causal pathways [32,33].

Gene expression profiling of the peripheral blood for stroke diagnosis

Structural DNA studies are being used to study the role of single nucleotide polymorphisms in vascular disease risk (see following text). Dynamic changes in DNA (as reflected by changes in messenger RNA) can now also be measured with gene expression profiling that permits the expression of thousands of genes to be measured simultaneously using microarray technology (also known as gene chip technology). Moore et al. [34••] have demonstrated a gene expression signature of acute ischemic stroke in peripheral blood mononuclear cells (lymphocytes and monocytes). Using Affymetrix (Santa Clara, CA) microarrays and a genome-wide scan across 22,283 gene probes, they identified a panel of 22 genes that were 80% specific for the diagnosis of acute ischemic stroke. Genes were related to hypoxic stress, to inhibition of neuronal apoptosis, and to the altered cerebral microenvironment (Table 3). This panel of

Table 3. Panel of 22 genes for stroke diagnosis*

Rank	UniGene ID	Gene name
Leukocyte activation/differentiation		
1	74076	CD163
2	178470	Hypothetical protein FLJ22662 Laminin A motif (probable adhesion)
5	279518	Amyloid beta (A4) precursor-like protein 2
6	64896	N-acetylneuraminate pyruvate lysase
7	25647	v-fos FBJ murine osteosarcoma viral oncogene homolog
8	439608	Toll-like receptor 2
12	444105	Ectonucleoside triphosphate diphosphohydrolase 1
13	434488	Chondroitin sulfate proteoglycan 2 (versican)
15	285115	Interleukin-13 receptor, alpha 1
18	75627	CD14 antigen
19	169998	Bone marrow stromal cell antigen 1/CD157
20	97199	Complement component 1, q subcomponent, receptor 1
21	122591	Paired immunoglobin-like type 2 receptor alpha
22	77424	Fc fragment of IgG, high affinity Ia, receptor for (CD64)
Hypoxia		
3	441047	Adrenomedullin
11	171695	Dual specificity phosphatase 1
14	88974	Cytochrome b-245, beta polypeptide (chronic granulomatous disease)
16	81118	Leukotriene A4 hydrolase
17	292477	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)
Vascular repair		
10	443120	CD36 antigen (thrombospondin receptor)
Possible response to altered cerebral microenvironment		
9	508565	Baculoviral IAP repeat-containing protein 1 (neuronal apoptosis inhibitory protein)
Unknown		
4	381058	KIAA0146 protein

*A panel of 22 genes derived from 22,283 gene probes was 78% sensitive and 80% specific for the diagnosis of acute ischemic stroke in two separate cohorts of patients and volunteers. The ranking was obtained from the statistical evaluation of the individual genes. (*Adapted from* Moore et al. [34••]; with permission.)

genes has the potential to be developed into a rapid diagnostic test for stroke (eg, a chip-based test). There was minimal overlap of the gene list with those of multiple sclerosis and sickle cell disease, suggesting specificity of the results for stroke. There was a partial dependence of the gene listing on vascular risk conditions, suggesting that it may be possible in the future to identify panels of genes that are indicative of an individual's future risk of atherosclerotic vascular disease. The peripheral blood is likely to reflect systemic changes associated with the body's adaptive response to ischemic stroke. Results were recently confirmed in part by Tang et al. [35••], adding further to the promise of this approach, which could also be used to develop panels of genes related to an individual's risk of developing a stroke.

Profiling of the peripheral blood proteome for stroke diagnosis

Panels of proteins also offer promise for stroke diagnosis. Lynch et al. [36••] have worked on developing a blood-based diagnostic test for stroke by evaluating the sensitivity, specificity, and reproducibility of 26 blood-borne markers believed to play a role in the ischemic cascade. Four were highly correlated with stroke ($P < 0.001$): one marker of glial activation (S100β), two markers of inflammation (MMP-9 and VCAM), and one marker of thrombosis (von Willebrand factor). In combination, this panel had a sensitivity of 90% and a specificity of 90% for predicting stroke. The authors concluded that a panel of blood-borne biochemical markers may be helpful in identifying patients with acute cerebral ischemia who could benefit from

urgent care, and in identifying stroke patients in the prehospital setting so that they could be put on a fast track to an institution equipped to care for patients with acute stroke. These results require validation in an independent patient cohort. The same group has evaluated other panels of proteins for stroke diagnosis [37]. The ongoing Biomarker Rapid Assessment of Ischemic Injury (BRAIN) study is evaluating the Triage Stroke Panel (Biosite, San Diego, CA) of a number of plasma markers, specifically, B-type natriuretic peptide (BNP), fibrin degradation products containing D-Dimer, MMP-9, and S-100β.

Advanced proteomics methods permit the study of the entire proteome to be carried out, ranging from the largest and most abundant proteins in the plasma to the smallest peptide fragments. The difficulty is with purifying and being able to recognize the proteins, especially using the serum surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF) proteomics methodology. Some authors have identified proteins in the cerebrospinal fluid and then applied this information to the serum [38,39•]. Allard et al. [38] identified PARK7 and nucleoside diphosphate kinase A as plasma markers for the early diagnosis of stroke. Using the SELDI-TOF methodology, the same group has reported that apolipoprotein (Apo) C-I and ApoC-III could be potential plasma markers to distinguish between ischemic and hemorrhagic stroke [39•].

Other cellular and molecular profiling approaches for prediction of stroke recurrence and death

Other cellular profiling approaches that have been tried include profiling of endothelial cells and endothelial cell microparticles using flow cytometry, and these have had promising results [40,41]. CD4+CD28- is a proinflammatory subset of T-cell lymphocytes that has been associated with an increased risk of stroke recurrence and death [42,43]. Whether these cells are a sign of an aged immune system or are actually pathogenic mediators is yet to be determined. Hematopoetic progenitor cells may also be of promise [44]. Ongoing studies are looking at the possible prognostic value of lipoprotein-associated phospholipase A_2 (Lp-PLA_2) in the prediction of stroke recurrence and death [45].

Stroke Risk

In the same way that acute stroke management relies heavily on clinical examination, the assessment of an individual's future risk of stroke relies heavily on the assessment of vascular risk factors such as the presence of hypertension and/or diabetes. However, vascular risk assessment is also imperfect at best; for example, the Framingham stroke risk score has an accuracy of around 60% to 80%. Dramatic improvements have occurred in the understanding of the molecular mechanisms underlying atherosclerosis in recent years, with the overall concept changing from one of a lipid storage disease to one of chronic inflammation [46]. Improved understanding of mechanisms involved in the vulnerable plaque has further opened up new avenues for finding imaging and molecular markers to identify individuals at greatest risk. Fibrinogen, hsCRP, PAI-1, periodontal disease, salivary lysozyme, and soluble CD40 ligand are recent possible markers studied, along with studies of single nucleotide polymorphisms of inflammatory and coagulation genes.

Biomarkers of stroke risk have progressed further than those for use in acute stroke management. In June 2005, the first biomarker for stroke risk associated with atherosclerosis, Lp-PLA_2, (an inflammatory enzyme) was approved by the FDA (the PLAC test; diaDexus, South San Francisco, CA). Results from the Atherosclerosis Risk in Communities (ARIC) study [47••] demonstrated that individuals with elevated levels of Lp-PLA_2 have a statistically significant twofold risk of suffering an ischemic stroke over a period of 6 to 8 years compared with individuals with low levels of Lp-PLA_2. These findings were independent of traditional risk factors such as systolic blood pressure, smoking status, and diabetes, as well as body mass index. As Lp-PLA_2 and systolic blood pressure levels are additive in their ability to predict stroke risk, the PLAC test can help identify hypertensive patients who are at the greatest risk of stroke. The clinical utility of this marker, however, has been questioned [15].

High-sensitivity CRP is close to being used as a biomarker of cardiovascular risk and provides a good example of the issues involved in developing a biomarker for clinical use. A recent review indicated that hsCRP is not ready for use as a test for predicting primary or secondary stroke [48•]. In the case of coronary artery disease, some preliminary recommendations have been made (of utility in the medium-risk patient) [49•] and the interventional Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial is in progress [50] to determine the clinical utility of hsCRP. There is growing interest in the use of applying the methods of genomics and proteins in studies of vascular risk assessment.

Conclusions

The search for blood-based biomarkers for stroke and for stroke risk has been going on for decades. The ideal properties of a biomarker must be borne in mind when interpreting study results. The first blood biomarker for stroke risk has now been approved by the FDA and a panel of proteins for early stroke diagnosis is in clinical trial. "Discovery approaches" also offer a most promising way to find new blood biomarkers for stroke. With these, panels of genes or proteins may be identified in the blood for stroke diagnosis and risk as opposed to laboriously trying out one biomarker at a time, but the specificity of blood panels has not been fully worked out. The ultimate

aim is that assays can be developed for rapid use (eg, in the ambulance) for the more accurate acute treatment of patients. Whether these will stand alone or be adjunctive to imaging methods remains to be seen. The most rapid progress could come from integrated, well-planned, multidisciplinary studies to check the accuracy of these panels, perhaps through consortiums or stroke drug trials. At present, biomarkers offer considerable promise for better risk prediction of stroke, but their value in diagnosis, treatment, and management of stroke, as well as their cost effectiveness, has yet to be established. Cost effectiveness may well be the deciding factor in the development and use of stroke biomarkers.

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A panel of 22 genes was more than 80% sensitive and specific for the diagnosis of stroke. The gene list was generated from a starting number of 22,283 gene probes. Results were validated with real time polymerase chain reactions and in an independent patient sample.

35.•• Tang Y, Xu H, Du X, et al.: **Gene expression in blood changes rapidly in neutrophils and monocytes after ischemic stroke in humans: a microarray study.** *J Cereb Blood Flow Metab* 2006, **26:**1089–1102.

Provided confirmatory evidence of Moore et al.'s [34••] results showing some overlap of gene listings and reproducibility of genes despite different blood collection systems. Gene expression changes were seen as early as 3 hours after onset and were remarkably constant over the first 24 hours of ischemic stroke. Gene expression changes seemed to come from the neutrophil and monocyte fractions, as opposed to the monocyte and lymphocyte fractions.

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A panel of four proteins was 90% sensitive and specific for the diagnosis of acute stroke. These results need to be validated, however. Of the 26 blood-borne markers analyzed, the four that were highly correlated with stroke ($P < 0.001$) were one marker of glial activation (S100β), two markers of inflammation (MMP-9 and VCAM), and one marker of thrombosis (von Willebrand factor).

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47.•• Ballantyne CM, Hoogeveen RC, Bang H, et al.: **Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study.** *Arch Intern Med* 2005, **165:**2479–2484.

Study demonstrated that individuals with elevated levels of Lp-PLA_2 had a statistically significant twofold risk of suffering an ischemic stroke over a period of 6 to 8 years compared with individuals with low levels of Lp-PLA_2. These findings were independent of traditional risk factors such as systolic blood pressure, smoking status, and diabetes, as well as body mass index. Because Lp-PLA_2 and systolic blood pressure levels were additive in their ability to predict stroke risk, the PLAC test can help identify stroke-prone hypertensive patients. The authors also concluded that Lp-PLA_2 and CRP levels may be complementary beyond traditional risk factors in identifying middle-aged individuals at increased risk for ischemic stroke, as individuals with high levels of both CRP and Lp-PLA_2 were at the highest risk after adjusting for traditional risk factors compared with individuals with low levels of both, whereas others were at intermediate risk.

48.• Di Napoli M, Schwaninger M, Cappelli R, et al.: **Evaluation of C-reactive protein measurement for assessing the risk and prognosis in ischemic stroke: a statement for health care professionals from the CRP Pooling Project members.** *Stroke* 2005, **36:**1316–1329.

Study concluded that at present there is not sufficient evidence to recommend measurement of CRP in the routine evaluation of cerebrovascular disease risk in primary prevention because there is insufficient evidence as to whether early detection, or intervention based on detection, improves health outcomes, although shared risk of cardiovascular disease indicates this may be of value. In secondary stroke prevention, elevated CRP does add to existing prognostic markers, but it remains to be established whether specific therapeutic options can be derived from this.

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The following recommendations were made for hsCRP as a risk marker in clinical practice for cardiovascular disease. 1) hsCRP is an independent marker of risk that may be used at the discretion of the physician in patients judged by global risk assessment to be at intermediate risk (10% to 20% risk for coronary heart disease per 10 years) or cardiovascular disease (CVD). hsCRP may help direct further evaluation and therapy in the primary prevention of CVD. The benefits of such therapy based on this strategy remain uncertain. 2) hsCRP is an independent marker of risk and may be used at the discretion of the physician as part of a global coronary risk assessment in adults without known CVD. The benefits of this strategy remain uncertain. 3) hsCRP levels may be useful in motivating patients to improve their lifestyle behaviors. The benefits of this strategy remain uncertain.

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