The Stroke Biospecimens, Biomarkers, and Laboratory Tests Subgroup recommended that stroke clinical studies consider collecting the Recommended biomarkers. They also listed other emerging biomarkers that studies may want to collect.

## Recommended biomarkers:

* + **Protein assays:**
    - Alipoprotein A (ApoA)
    - Alipoprotein B (ApoB)
    - Brain natriuretic peptide (BNP)
    - C-reactive protein (CRP)
    - D-dimer
    - Fibrinogen
    - Lipoprotein(a) [Lp(a)]
    - Lipoprotein-associated phospholipase A2 (Lp-PLA2)
    - Troponin
    - Von Willebrand factor (VWF)
  + **Genotyping assays:**
    - Cytochrome P450 2C19 (CYP2c19)
    - Factor V Leiden
  + **Metabolite assays:**
    - Homocysteine (homoCYS)
    - Uric acid

## Emerging biomarkers:

* + **Protein assays:**
    - Inter-Cellular Adhesion Molecule 1 (ICAM-1)
    - Interleukin-6 (IL-6)
    - Matrix-metalloproteinase (MMP)
    - Neuron-specific enolase (NSE)
    - Plasminogen activator inhibitor type 1 (PAI)
    - P-selectin
    - S100 calcium binding protein B (S-100B)
    - Soluble CD40 ligand (sCD40 ligand)
    - Tumor necrosis factor (TNF)
    - Vascular cell adhesion molecule 1 (VCAM-1)

## Genotyping assays:

* + - Activated Protein C Resistance (APC-R)
    - Angiotensin converting enzyme (ACE)
    - Alipoprotein E (ApoE)
    - E-selectin
    - Phosphodiesterase 4D (PDE4D)
    - Prothrombin G20210A

The subsequent pages provide a short synopsis on each of the Recommended biomarkers.

## Alipoprotein A (ApoA)

### Type of Assay

Protein

### Annotation

Apo A-I is thought to be anti-atherogenic. It transports high density lipoprotein. ApoB-to-Apo A I ratio is associated with carotid plaque and with fatal and nonfatal ischemic stroke. One potential practical advantage of this ratio over conventional lipid markers, like LDL cholesterol, is that fasting is not be required for a reliable assessment. Apo A-I has also been associated with young adult stroke in some series (Erquo et al 2010).

### References

1. Gardener, H., Morte, D.D., Elkind, M., Sacco, R.L., Rundek, T. (2009). Lipids and carotid plaque in the Northern Manhattan Study (NOMAS). BMC Cardiovascular Disorders, 9, 55-63.
2. Holme, I., Aastveit, A.H., Niklas, H., Jungner, I., Walldius, G. (2009). Inflammatory markers, lipoprotein components and risk of major cardiovascular events in 65,005 men and women in the Apolipoprotein MOrtality RISk study (AMORIS). J Internal Med., 265, 275-287.
3. Walldius G, Aastveit AH, Jungner I. (2006). Stroke mortality and the apoB/apoA-I ratio: Results of the AMORIS prospective study. J Intern Med., 259, 259-266.
4. Di Angelantonio E, Sarwar N., Perry P., Kaptoge, S., Ray, K.K. et al. (2009) Major Lipids, Apolipoproteins, and Risk of Vascular Disease Jama, 302(18), 1993-2000.
5. Erqou S, Thompson A, Di AE et al. (2010). Apolipoprotein(a) isoforms and the risk of vascular disease: systematic review of 40 studies involving 58,000 participants. J Am Coll Cardiol, 11(19), 2160-2167.

## Alipoprotein B (ApoB)

### Type of Assay

Protein

### Annotation

ApoB is thought to be atherogenic. ApoB is a transporter of very low density lipoprotein, intermediate-density lipoprotein and LDL particles. ApoB-to-Apo A-I ratio is associated with carotid plaque and with fatal and nonfatal ischemic stroke. One potential practical advantage of this ratio over conventional lipid markers, like LDL cholesterol, is that fasting is not be required for a reliable assessment. Apo B levels are reduced by statin use. Apo B is predictive of ischemic stroke in patients with previous transient ischemic attack. It has also been associated with young adult stroke and ApoB/ApoA is an independent predictor (Sabino 2008).

### References

1. Gardener, H., Morte, D.D., Elkind, M., Sacco, R.L., Rundek, T. (2009). Lipids and carotid plaque in the Northern Manhattan Study (NOMAS). BMC Cardiovascular Disorders, 9, 55-63.
2. Holme, I., Aastveit, A.H., Niklas, H., Jungner, I., Walldius, G. (2009). Inflammatory markers, lipoprotein components and risk of major cardiovascular events in 65,005 men and women in the Apolipoprotein MOrtality RISk study (AMORIS). J Internal Med., 265, 275-287.
3. Bhatia M, Howard SC, Clark TG, Neale R, Qizilbash N, Murphy MFG, Rothwell PM (2006). Apolipoproteins as Predictors of Ischaemic Stroke in Patients with a Previous Transient Ischaemic Attack. Cerebrovasc Dis 2006;21:323-328.
4. Sabino AP, De Oliveira SM, Moreira LL et al. (2008). ApoB/ApoA-I ratio in young patients with ischemic cerebral stroke or peripheral arterial disease. Transl Res, 152(3),113-118.

## Brain natriuretic peptide (BNP)

### Type of Assay

Protein

### Annotation

Brain natriuretic peptide (BNP) is a sensitive marker of cardiac contractile dysfunction. Levels of BNP have been shown to predict atrial fibrillation. BNP has been measured subacutely in patients with ischemic stroke, and levels associated with cardioembolic stroke mechanism and atrial fibrillation. Levels of BNP are also increased in congestive heart failure and renal failure.

### References

No references

## C-reactive protein (CRP)

### Type of Assay

Protein

### Annotation

High sensitivity C-reactive protein (hsCRP) is an inflammation-related biomarker related to risk of stroke in several studies. A meta-analysis including 54 prospective cohort studies (n=160,309) found an increased risk of ischemic stroke (RR per SD logCRP 1.44, 95% CI 1.32-1.57) adjusted for age and sex; this attenuated (RR 1.27, 95% CI 1.15-1.40) when further adjusted for other risk factors. Those with hsCRP>2 mg/L and <1 other risk factor may benefit from treatment with rosuvastatin or other statins, based on the results of a large RCT. CDC/AHA guidelines recommend considering measuring hsCRP for primary preventive purposes only in patients at intermediate risk of vascular disease. The role of hsCRP in secondary prevention after stroke remains uncertain, though some studies suggest hsCRP predicts mortality and recurrent events. Furthermore, hsCRP is acutely elevated in children with stroke that is associated with non-moyamoya arteriopathy (Bernard et al 2010).

### References

1. The Emerging Risk Factors Collaboration. (2010). C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet, 375, 132-140.
2. Everett, B.M., Glynn, R.J., MacFadyen, J.G., Ridker, P.M. (2010). Rosuvastatin in the Prevention of Stroke Among Men and Women With Elevated Levels of C-Reactive Protein: Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER). Circulation, 121, 143-150.
3. Pearson, T.A., Mensah, G.A., Alexander, R.W., et al. (2003). Markers of inflammation and cardiovascular disease. Application to clinical and public health practice. A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation, 107, 499-511.
4. Di Napoli, M., Schwaninger, M., Cappelli, R., et al. (2005). 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, 36, 1316-1329.
5. Bernard, T.J., et al., Biomarkers of hypercoagulability and inflammation in childhood-onset arterial ischemic stroke. J Pediatr., 156(4), 651-656.

## Cytochrome P450 2C19 (CYP2c19)

### Type of Assay

Genotyping

### Annotation

This test is only FDA approved in the context of its inclusion in the Roche AmpliChip P450, which allows for the identification of genetic variants in CYP2d6 and CYP2c19 genes. Genetic variation in 2c19 allows for the identification of poor metabolizers of medications that are metabolized through the 2c19 pathway, including anti-epileptics, anti-convulsants, and psychotherapeutic drugs. Although CYP2c19 variants can also identify poor metabolizers of Clopidogrel (Plavix), there is no specific mention of this intended use.

### References

DNA Direct. (n.d.) Drugs Metabolized by CYP450. Retrieved September 15, 2010

## D-dimer

### Type of Assay

Protein

### Annotation

As products of fibrinolysis, serum D-dimer concentrations are acutely elevated in adult ischemic stroke patients, but serum D-dimer concentrations do not independently have the necessary sensitivity nor specificity to singularly serve as a reliable diagnostic marker (Haapaniemi 2009). In other studies, D-dimer levels have been associated with early clinical deterioration, stroke severity, poor clinical outcomes in patients with both ischemic and hemorrhagic stroke. However, D-dimer levels alone lack the robustness to serve as independent predictors of these endpoints (Barber 2006; Delgado 2006). D-dimer is elevated in acute childhood stroke and decreases over time in non-cardioembolic stroke (Bernard et al 2010). It is persistently elevated in childhood cardioembolic stroke (Bernard et al 2010).

### References

1. Bernard, T.J., Fenton, L.Z., Apkon, S.D., Broda, R., Wilkening, G.N., et al. (2009) Biomarkers of hypercoagulability and inflammation in childhood-onset arterial ischemic stroke. J Pediatr., 156(4), 651-656.
2. Haapaniemi, E., Tatlisumak, T. (2009). Is D-dimer helpful in evaluating stroke patients? A systematic review. Acta Neurol Scand., 119, 141-150.
3. Barber, M., Langhorne, P., Rumley, A., et al. (2006). D-dimer predict early clinical progression in ischemic stroke. Stroke, 37, 1113-1115.
4. Delgado, P., Alverez-Sabin, J., Abilleira, S., et al. (2006). Plasma D-dimer predicts poor outcome after acute intracerebral hemorrhage. Neurology, 67, 94-98.

## Fibrinogen

### Type of Assay

Protein

### Annotation

Controlling for diabetes and hypertension, higher fibrinogen levels are associated with the development of ischemic stroke (Fibrinogen Studies Collaboration 2005). After controlling for age and initial stroke severity, higher initial fibrinogen levels have been associated with poor outcomes in adults (del Zoppo et al 2009). Fibrinogen is also associated with childhood stroke that is associated with arteriopathy and cardiac disorders (Kopyta et al 2010).

### References

1. Kopyta, I., Sarecka-Hujar, B., Emich-Widera, E., Marszal, E., Zak, I. (2010). Association between lipids and fibrinogen levels and ischemic stroke in the population of the Polish children with arteriopathy and cardiac disorders. Wiad Lek, 63(1), 17-23.
2. Fibrinogen, S.C. (2005). Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an individual participant metaanalysis. JAMA., 294, 1799 –1809.
3. del Zoppo, G., Levy, D.E., Wasiewski, W.W., et al. (2009). Hyperfibrinogenemia and functional outcome from acute ischemic stroke. Stroke, 40, 1687-1691.

## Factor V Leiden

### Type of Assay

Genotyping

### Annotation

The factor V (Leiden) mutation (1691G→A) occurs primarily in Caucasian populations and is a major risk factor for venous thrombosis and a lesser risk factor for arterial thrombosis. Factor V mutation analysis is also recommended to confirm positive APCR (activated Protein C resistance) tests. It is associated with stroke in childhood (Barrierinho et al, 2003; Duran et al, 2005; Kenet et al, 2007; Nowak-Gottl et al, 1998).

### References

1. Barreirinho, S., Ferro, A., Santos, M., et al. (2003) Inherited and acquired risk factors and their combined effects in pediatric stroke. Pediatr Neurol., 28(2), 134-138.
2. Duran, R., Biner, B., Demir, M., Celtik, C., Karasalihoglu, S. (2005). Factor V Leiden mutation and other thrombophilia markers in childhood ischemic stroke. Clin Appl Thromb Hemost, 11(1), 83-88.
3. Kenet, G., Sadetzki, S., Murad, H., et al. (2000). Factor V Leiden and antiphospholipid antibodies are significant risk factors for ischemic stroke in children. Stroke, 31(6), 1283-1288.
4. Nowak-Gottl, U., Strater, R., Heinecke, A., et al. (1999). Lipoprotein (a) and genetic polymorphisms of clotting factor V, prothrombin, and methylenetetrahydrofolate reductase are risk factors of spontaneous ischemic stroke in childhood. Blood, 94(11), 3678-3682.

## Homocysteine (homoCYS)

### Type of Assay

Metabolite

### Annotation

Extensive data supports a role for hyperhomocysteinemia as an independent risk factor for cerebrovascular atherosclerosis, leukoaraiosis, and ischemic stroke. Levels of homocysteine can be affected by folate or vitamin B12 deficiency, metabolic derangements, and many medications. In addition, levels of homocysteine increase after protein-rich meals. Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme involved in degradation of plasma homocysteine. A common MTHFR substitution, 677C→T, causes reduced enzyme activity and increases plasma levels of homocysteine. Despite strong epidemiological data linking homocysteine to the pathogenesis of cerebrovascular disease, two secondary stroke prevention studies targeting homocysteine have failed to show a benefit to vitamin therapy. Homocysteine levels have been associated with stroke in children (Ganesan et al, 2003). MTHFR is no longer recommended as a first line test as homocysteine is preferred.

### References

1. Ganesan, V., Prengler, M., McShane, M.A., Wade, A.M., Kirkham, F.J. (2003). Investigation of risk factors in children with arterial ischemic stroke. Ann Neurol., 53(2), 167-73.

## Lipoprotein(a) [Lp(a)]

### Type of Assay

Protein

### Annotation

Lp(a) consists of a protein moiety, apolipoprotein B-100 (LDL) linked, on a 1:1 molar basis, by a disulfide bond to apolipoprotein (a). Lp(a) decreases systemic fibrinolytic activity through multiple mechanisms. Lp(a) inhibits binding of plasminogen to annexin, preventing the conversion of plasminogen to plasmin. Lp(a) increases circulating levels of plasminogen activator inhibitor (PAI), thus lowering levels of tPA. Epidemiological studies in cerebrovascular disease have provided conflicting evidence regarding the role lp(a) plays in stroke risk, recurrence, or outcome. Published studies have used different assays, making comparisons difficult. Aspirin and niacin, commonly used in a stroke-prone population, can affect lp(a) levels. Lp(a) has been associated with stroke in children with cardiac disease (Strater et al, 1999). It has also been associated with recurrent stroke in childhood (Strater et al, 2002). Furthermore, it has shown to be a risk factor for stroke (Nowak-Gottl et al, 1999).

### References

1. Strater, R., Vielhaber, H., Kassenbohmer, R., von, K.R., Gobel, U., Nowak-Gottl, U. (1999). Genetic risk factors of thrombophilia in ischaemic childhood stroke of cardiac origin. A prospective ESPED survey. Eur J Pediatr., 158(Suppl 3), S122-S125.
2. Strater, R., Becker, S., von, E.A., et al. (2002). Prospective assessment of risk factors for recurrent stroke during childhood--a 5-year follow-up study. Lancet, 360(9345), 1540-1545.
3. Nowak-Gottl, U., Strater, R., Heinecke, A., et al. (1999). Lipoprotein (a) and genetic polymorphisms of clotting factor V, prothrombin, and methylenetetrahydrofolate reductase are risk factors of spontaneous ischemic stroke in childhood. Blood, 94(11), 3678-3682.

## Lipoprotein-associated phospholipase A2 (Lp-PLA2)

### Type of Assay

Protein

### Annotation

Lp-PLA2 is a macrophage-derived enzyme involved in metabolism of LDL that is responsible for release of inflammatory mediators. Relative elevations in serum levels of Lp-PLA2 are associated with increased risk of incident ischemic stroke, independent of the effect of hsCRP. A large meta-analysis found evidence that LpPLA2 predicts risk of a first stroke. LP-PLA2 may also predict risk of recurrent stroke and other vascular events after a first stroke.

### References

1. Oei, H.H., van der Meer, I.M., Hofman, A., et al. (2005). Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation, 111, 570-575.
2. Ballantyne, C.M., Hoogeveen, R.C., Bang, H., et al. (2005). 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., 165, 2479-2484.
3. Lp-PLA(2) Studies Collaboration, Thompson, A., Gao, P., Orfei, L., Watson, S., (2010). Lipoprotein-associated phospholipase A2 and the risk of coronary disease, stroke and non-vascular mortality. Individual data analysis of 78,989 participants from 32 prospective studies. Lancet, 37, 1536-1544.
4. Elkind, M.S.V., Tai, W., Coates, K., Paik, M.C., Sacco, R.L. (2006). Lipoprotein-associated phospholipase A2, C-reactive protein, and outcome after ischemic stroke. Arch Int Med., 166, 2073-2080.

## Troponin

### Type of Assay

Protein

### Annotation

Cardiac troponins classically are associated with acute coronary syndrome and heart failure. Elevations in troponin have also been observed after stroke. This post-stroke elevation in cardiac troponins is associated with sympathoadrenal activation and is associated with electrocardiogram changes suggestive of acute coronary syndrome and with death. Troponin T has been associated with large infarcts involving the MCA distribution. Troponin I elevations have been associated with new onset atrial fibrillation soon after stroke.

### References

1. Kerra, G., Raya, G., Wub, O., Stotta, D.J., Langhornea, P., (2009). Elevated Troponin after Stroke: A Systematic Review. Cerebrovascular Diseases, 28(3), 220-226.
2. Bugnicourta, J.M., Rogezb, V., Guillaumontc, M.P., Rogezd, J.C., Canapleb, S., et al. (2010). Troponin Levels Help Predict New-Onset Atrial Fibrillation in Ischaemic Stroke Patients: A Retrospective Study Euro Neuro, 63, 24-28
3. Jensen, J.K., Kristensen, S.R., Bak, S., Atar, D., Høilund-Carlsen, P.F., Mickley, H. (2007). Frequency and significance of troponin T elevation in acute ischemic stroke. Am J Cardiol., 99, 108-112
4. Chalela, J.A., Ezzeddine, M.A., Davis, L., Warach, S. (2004). Myocardial injury in acute stroke A troponin I study Neurocritical Care, 1, 343-6

## Uric acid

### Type of Assay

Metabolite

### Annotation

Uric acid, a product of purine metabolism, is fairly abundant in human plasma, with normal concentrations ranging from 3.5 mg/dL to 8.3 mg/dL, and serves as one of the most important antioxidants in plasma. Compared to other biomarkers presented here, less data are available for uric acid in acute stroke. Increasing levels of uric acid have been associated with increasing risk of ischemic stroke and acute myocardial infarction (Bos 2006). In the setting of acute ischemic stroke, uric acid levels are increased and decline gradually over the course of days post-stroke. Declines in uric acid levels have been associated with stroke progression, increased morbidity and mortality (Brouns 2010). The potential neuroprotective effect of uric acid, serving as an antioxidant, in the setting of acute ischemic stroke is currently under study (Amaro 2007).

### References

1. Bos, M.J., Koudstaal, P.J., Hofman, A., et al. (2006). Uric acid is a risk factor for myocardial infarction and stroke: the Rotterdam study. Stroke, 37, 1503-1507.
2. Brouns, R., Wauters, A., Van De Vijver, G. (2010). Decrease in uric acid in acute ischemic stroke correlates with stroke severity, evolution and outcome. Clin Chem Lab Med., 48, 383-390.
3. Amaro, S., Soy, D., Obach, V., et al. (2007). A pilot study of dual treatment with recombinant tissue plasminogen activator and uric acid in acute ischemic stroke. Stroke, 38, 2173-2175.

## Von Willebrand factor (VWF)

### Type of Assay

Protein

### Annotation

Megakaryocytes and endothelial cells synthesize von Willebrand factor. Clot stabilization is achieved once vWF becomes activated at the site of vascular injury, leading to binding of fibrin, transglutamination by factor XIII, and covalent crosslinking. vWF mediates the incorporation of platelets into thrombus. Elevations in von Willebrand factor have been associated with an increased risk of ischemic stroke. Levels of vWF have been shown to be higher in patients with a greater burden of small vessel/lacunar strokes. vWF levels are also associated with many stroke risk factors such as atrial fibrillation, high grade carotid stenosis, and hyperhomocysteinemia. Single nucleotide polymorphisms in the promoter region of the vWF gene, affecting vWF levels, have been identified.

### References

No references