Hypercoagulation Testing in Ischemic Stroke

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Context.—The utility of laboratory testing for hypercoagulability in the setting of stroke is uncertain.

Objective.—To review the current literature and to make recommendations with regard to laboratory testing for various hypercoagulability risk factors for ischemic stroke.

Data Sources.—Published articles studying the utility of various hypercoagulation tests in predicting initial and/or recurrent stroke or transient ischemic attack as well as cerebral vein thrombosis were collected and reviewed, with an emphasis on prospective studies.

Laboratory hypercoagulation testing for thrombosis in the cerebrovascular system, in particular, arterial ischemic stroke, transient ischemic attack (TIA), or cerebral venous thrombosis, are discussed in this review. The risk of stroke with elevated low-density lipoprotein cholesterol, decreased high-density lipoprotein cholesterol, or other lipid abnormalities is not as well understood as it is for myocardial infarction. Other tests are emerging as potentially useful markers for risk of recurrence. C-reactive protein (CRP) may predict future risk of stroke in men and women, similar to the apparent association between CRP and the risk of myocardial infarction. Data are also accumulating regarding the significance of elevated homocysteine or lipoprotein(a), abbreviated Lp(a), in patients with arterial ischemic stroke. Testing for antiphospholipid antibodies (lupus anticoagulants and antカードoliphin antibodies) may also be useful in certain patients. Tests for the prothrombin G20210A or factor V Leiden mutation and protein C, protein S, or antithrombin deficiencies can be informative for patients with cerebral venous thrombosis, but the utility of these tests with arterial ischemic stroke may be limited. These laboratory tests are discussed in greater detail in the following sections, with pertinence to their ability to predict neurologic thromboses as evidenced mainly by prospective studies. Recommendations are made with regard to laboratory testing for risk factors for ischemic stroke, based on evidence in the literature and findings from a prior literature review with expert consensus (Table).

Conclusions.—Certain tests, such as C-reactive protein, homocysteine, antiphospholipid antibodies, and lipoprotein(a), may be useful in patients with a history of stroke or at high risk for stroke, as evidenced by prospective data. Factor V Leiden, prothrombin G20210A, protein C, protein S, and antithrombin are not recommended for routine testing but may be useful in certain populations, such as in pediatric patients or in patients with cerebral vein thrombosis.

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ANTIPHOSPHOLIPID ANTIBODIES (LUPUS ANTICOAGULANT AND/OR ANTICARDIOLIPIN ANTIBODIES)

Antiphospholipid antibodies are acquired autoantibodies directed against phospholipids or phospholipid-protein complexes and are associated with an increased risk of both venous and arterial thrombosis. Testing for antiphospholipid antibodies should include tests for the lupus anticoagulant, as well as tests for both immunoglobulin (Ig) G and IgM subclasses of antカードoliphin antibodies. The majority of studies investigating the relationship between antiphospholipid antibodies and the risk of thrombosis have been retrospective.

Among prospective studies of initially healthy individuals, 1 study of 360 individuals with antiphospholipid antibodies (lupus anticoagulants and/or antカードoliphin antibodies) found that 4.7% of individuals developed venous thrombosis, whereas 4.7% developed arterial thrombosis during a median follow-up of 3.9 years. All but 1 of the arterial thrombotic events were either strokes or TIsAs. The risk for any thrombosis was greater among those with a history of prior thrombosis or those who had at least moderately elevated levels of IgG antカードoliphin antibodies greater than 40 IgG phospholipid units (GPL). It is important to note that results are not interchangeable among the different assays that are available, and a cutoff of 40 GPL, for example, does not apply to all types of assays. In a prospective study evaluating the association between antカードoliphin antibodies and first-time stroke in men, Brey et al found that IgG antカードoliphin antibodies were more common in men who later developed stroke than in healthy patients (adjusted relative odds of 2.2 at 15 years and 1.5 at 20 years of follow-up, both statistically significant). The same findings were not true for IgM antカードoliphin antibodies.

Other prospective studies of initially healthy individuals have yielded somewhat conflicting results. One study showed no association between baseline IgG antカードoliphin antibodies and risk of future stroke, whereas another...
found that IgM, but not IgG or IgA, anticardiolipin antibodies were significantly more common among individuals who subsequently developed ischemic stroke or cerebral hemorrhage than in individuals who remained healthy. In the latter study, the association with IgM anticardiolipin antibodies was no longer significant once adjustment was made for other risk factors. Yet another prospective study, which collectively analyzed levels of IgG, IgM, and IgA anticardiolipin antibodies, showed that elevated concentrations of these antibodies significantly predicted risk of future stroke or TIA in women but not in men.19 Prospective studies have also been performed in individuals with a history of stroke or with stroke at the time of study initiation. Nencini and colleagues followed 10 stroke patients with lupus anticoagulants and/or anticardiolipin antibodies and 49 stroke patients without such antibodies during a mean follow-up period of 35 months. Stroke patients with antiphospholipid antibodies had a significantly higher rate of future stroke or other thrombosis during the follow-up period. Levine and colleagues, in another prospective study, followed 132 patients with anticardiolipin antibodies and stroke or TIA for a mean of nearly 2 years. Patients with IgG anticardiolipin antibody titers greater than 40 GPL had more frequent subsequent events (stroke, TIA, other arterial or venous thrombosis, or death) as compared with patients with lower anticardiolipin antibody titers, and the events occurred earlier in affected patients. An earlier prospective study by some of the same authors found a similar association for IgG anticardiolipin antibodies and subsequent events, but not for IgM anticardiolipin antibodies, in which 81 patients with anticardiolipin antibodies and stroke or TIA were followed during a mean period of 3 years.10

Other studies have generated somewhat conflicting data. For example, in a prospective observational study of 300 patients with acute ischemic stroke, unadjusted mortality rates (but not rates of recurrent stroke, myocardial infarction, and vascular death) were significantly increased among patients with elevated IgG anticardiolipin antibodies (>20–40 GPL). However, the excess mortality associated with elevated IgG anticardiolipin antibodies was eliminated after adjustment for age, cardiovascular risk factors, and malignancy.11 Zielinska et al studied 194 stroke patients in whom IgG anticardiolipin antibodies were significantly associated with cognitive impairment at 30 days and 1 year of follow-up but not with recurrent stroke or mortality. The Antiphospholipid Antibodies and Stroke Study Group also showed no increased risk of recurrent stroke, TIA, other thrombosis, or death in 219 individuals with stroke and anticardiolipin antibodies during a median follow-up period of 2 years; however, the authors believed that the study lacked sufficient power to detect such a difference. A study of 128 young adults aged 18 to 45 with TIA or stroke showed no association between the presence of lupus anticoagulant or anticardiolipin antibodies of the IgG or IgM subclasses and subsequent thrombotic events, including stroke or TIA, during 3 years of follow-up. Also, a study of 185 children who survived a stroke or TIA found no association between persistently elevated IgG anticardiolipin antibody titers (assessed by at least 2 titers of 15 GPL measured ≥6 weeks apart) and risk of subsequent cerebrovascular events during 3 years of follow-up. Again, it is important to note that a cut-off of 15 GPL does not apply to all the different assays that are available. Finally, Levine et al followed a large prospective cohort of 1770 adult individuals for more than 2 years and found no increased risk of thrombo-occlusive events (including stroke, TIA, myocardial infarction, deep venous thrombosis, and pulmonary embolus) in patients with antiphospholipid antibodies. However, the authors likely overestimated the number of

### Recommended Laboratory Testing for Hypercoagulability Risk Factors in Ischemic Stroke

<table>
<thead>
<tr>
<th>Laboratory Test(s)</th>
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| Antiphospholipid antibodies (including lupus anticoagulant and/or anticardiolipin antibodies) | Consider in:  
* Patients with history of stroke  
* Patients with cerebral vein thrombosis |
| C-reactive protein | Consider in:  
* Patients at risk for stroke  
* Patients with history of cerebrovascular disease to assist in risk assessment for recurrent events |
| Homocysteine | Consider in:  
* Patients at high risk for stroke  
* Patients with history of stroke, atherosclerosis or cerebral vein thrombosis |
| Methylenetetrahydrofolate reductase C677T homozygous mutation (MTHFR 677TT) Lipoprotein(a) | Not recommended for routine testing; no prospective data linking homozygosity to increased risk of stroke  
* Patients with history of stroke  
* Pediatric patients with stroke  
* Patients with cerebral vein thrombosis |
| Prothrombin G20210A* | Consider in:  
* Pediatric patients with stroke  
* Patients with cerebral vein thrombosis |
| Factor V Leiden (activated protein C resistance)* | Consider in:  
* Pediatric patients with stroke  
* Patients with cerebral vein thrombosis |
| Protein C, protein S, or antithrombin deficiencies* | Consider in:  
* Young patients with stroke  
* Patients with personal or family history of venous thrombosis or paradoxical emboli  
* Patients with cerebral vein thrombosis |

* Recommendations based mainly on retrospective data.
subjects with antiphospholipid antibodies (n = 720; 40%) because a lupus anticoagulant was deemed present based on the positive results of only 1 screening test for lupus anticoagulant, even if the confirmatory test result was negative. The screening test lacks specificity compared with the confirmatory test.

All of the cited studies involved testing for lupus anticoagulants and/or IgG and IgM subclasses of anticardiolipin antibodies, tests that are standard in most laboratories. Newer and less-well-studied tests for antiphospholipid antibodies, such as anti-\( \beta_2 \)-glycoprotein I antibodies, antiprothrombin antibodies, or antiphosphatidylserine antibodies, would require prospective studies on stroke patients before recommendations could be made regarding their use. Nevertheless, a recent consensus meeting has suggested adding anti-\( \beta_2 \)-glycoprotein I to the list of laboratory tests that define antiphospholipid antibody syndrome.

Although there are few studies that investigate the association of cerebral venous thrombosis and antiphospholipid antibodies, a consensus of experts agreed that these tests may be useful in patients with cerebral venous thrombosis, based partly on extrapolation from other venous thrombosis studies.

In summary, antiphospholipid antibodies are associated with arterial as well as venous thrombosis. Strokes appear to be the most common type of arterial thrombosis associated with these antibodies, and some prospective studies suggest that the risk of stroke is increased by elevated anticardiolipin antibody titers, but the data are conflicting. Because 8 of the 14 prospective stroke or TIA studies described previously identified a significant finding in association with an antiphospholipid antibody, it would be recommended that testing for antiphospholipid antibodies (both lupus anticoagulant and anticardiolipin antibodies) be considered in patients with stroke and, in addition, in patients with cerebral venous thrombosis.

C-REACTIVE PROTEIN

C-reactive protein is an acute phase reactant that was initially discovered by interactions of the serum of patients recovering from pneumococcal infections with the C-polysaccharide of\textit{Pneumococcus}. C-reactive protein is elevated in a number of stressful and inflammatory states that occur following infection, injury, or trauma. The measurement of CRP via high-sensitivity CRP assays may add to the predictive value of the serum lipid profile for identifying individuals at risk for future cardiovascular events.

The relationship between elevated plasma CRP and ischemic stroke or TIA is less well studied, but more data continue to emerge.

The best evidence for the association between elevated levels of CRP and stroke comes from prospective studies. A prospective evaluation of the Framingham Study original cohort identified 591 men and 871 women with a mean age of 69.7 years who were free of stroke or TIA at baseline. During 12 to 14 years of follow-up, it was found that men in the highest CRP quartile had twice the risk and women had 2.7 times the risk of stroke or TIA, as compared with subjects in the lowest CRP quartile. The trend in risk across quartiles was statistically significant for both men and women, even after adjustment for smoking, total cholesterol, high-density lipoprotein cholesterol, systolic blood pressure, and diabetes. In a prospective evaluation of the Cardiovascular Health Study participants, 5417 subjects aged 65 years or older were followed for 10.2 years. Study subjects in the highest CRP quartile had a significantly greater adjusted risk of ischemic stroke versus those in the lowest quartile. Ridker and colleagues followed 122 apparently healthy women for 3 years who later developed a cardiovascular event and compared this group with 244 age- and smoking-matched controls. They found that levels of CRP in the highest quartile were associated with a 7-fold increased risk of myocardial infarction or stroke. The risk of future vascular events increased with each increasing quartile of CRP, a trend that was statistically significant. Finally, Curb et al looked specifically at the association between elevated CRP and thromboembolic stroke in apparently healthy men and found that the age-adjusted odds of stroke was 3.8-fold greater in the top versus bottom CRP quartile after 10 to 15 years of follow-up and 2.5-fold greater after 15 to 20 years of follow-up.

C-reactive protein has also been prospectively studied as a risk factor in patients with stroke or TIA at the time of study initiation. Among 228 patients with ischemic stroke, patients with a CRP more than 10.1 mg/L had significantly worse survival than those with CRP \( \leq \) 10.1 mg/L during 2.6 median years of follow-up, with cardiovascular causes accounting for 76% and 63% of the deaths in each group, respectively. A prospective study of 128 individuals with ischemic stroke in whom the CRP was measured within 24 hours after stroke showed that the probability of death or a new vascular event occurring during 1 year of follow-up was greater in those with a CRP in the highest tertile (54.8%) versus those with a CRP in the lowest tertile (12.1%), a finding that was statistically significant. Another prospective study of 71 stroke or TIA patients with intracranial large-artery occlusive disease as detected by transcranial Doppler ultrasonography and angiography showed that patients in the highest CRP quintile had a significantly greater risk for subsequent cerebral ischemic events or myocardial infarction as compared with those in the lowest CRP quintile during 1 year of follow-up.

Testing for CRP in the acute setting of stroke may be complicated by the fact that it is an acute phase reactant and could be elevated as part of the acute phase response in the setting of a thrombotic event. This raises the issue of how relevant CRP measurements taken during the acute setting of ischemic stroke are to subsequent risk of thrombotic events. Winbeck and colleagues investigated early serial CRP measurements in the acute setting of ischemic stroke and found that a CRP level measured within the first 12 hours after symptom onset was not an independent marker of long-term prognosis, whereas an elevated CRP measurement drawn between 12 and 24 hours of symptom onset was an independent predictor of poor outcome, being significantly associated with an increased incidence of cerebrovascular and cardiovascular events at 1 year of follow-up in 127 first-time stroke patients. In another study of 128 patients, Di Napoli et al measured CRP measured at different stages after acute stroke; they found that CRP measured at hospital discharge (mean \( \pm \) SD, 12 \( \pm \) 5 days) was the strongest independent marker of adverse outcome at 1 year, defined as either a new vascular event or death, as compared with measurements taken within 1 to 3 days after admission. The authors hypothesize that elevated CRP at hospital discharge reflects a persistent pathogenic inflammatory response in certain stroke patients and suggest that CRP measured at the time of hospital discharge may be a useful predictor of outcome.
of hospital discharge may in fact be of greater utility for risk stratification in patients with stroke.\(^{31}\) In addition, there is evidence that levels of CRP are stable during longer periods. In a study evaluating within-person fluctuations of CRP, paired samples obtained an average of 12 years apart in 379 patients showed a similar degree of variability to measurements of blood pressure and total serum cholesterol during the same period,\(^ {32}\) suggesting that CRP is sufficiently stable for potential use as a long-term predictor of vascular risk.

It has been theorized that patients with elevated CRP may benefit from therapy aimed at diminishing the inflammatory response and reducing levels of plasma CRP.\(^ {33}\) In a prospective observational study, Di Napoli and Papa\(^ {34}\) examined the effect of treatment with angiotensin-converting enzyme inhibitors in 507 patients with first-time ischemic stroke in terms of the relationship with CRP and outcome. Angiotensin-converting enzyme inhibitor therapy at the time of acute stroke was associated with lower median CRP levels and reduced 2-year cardiovascular risk as compared with a different blood pressure lowering regimen. The Pravastatin Inflammation/CRP Evaluation (PRINCE) study, a prospective, randomized, double-blind trial of 1702 men and women with no history of cardiovascular disease, found that participants receiving 40 mg/d of pravastatin had significantly lower median CRP levels at 12 and 24 weeks of therapy compared with participants receiving placebo, an effect that was independent of low-density lipoprotein levels.\(^ {35}\) A similar reduction in CRP levels was seen in 1182 patients with known cardiovascular disease treated with pravastatin.\(^ {36}\) Another prospective study examining aspirin therapy in 543 apparently healthy men with elevated baseline CRP levels showed that aspirin use was associated with significant reductions in the risk of myocardial infarction among men in the highest CRP quartile but only small, nonsignificant, reductions in men in the lowest quartile.\(^ {37}\) There is also evidence that elevated CRP may identify a group of patients without hyperlipidemia who would still benefit from statin therapy as primary prevention to decrease the risk for future acute coronary events.\(^ {38}\) To our knowledge, similar prospective direct intervention data have not yet been reported on the use of anti-inflammatory therapy and stroke outcome in the setting of elevated CRP, although results of an ongoing trial may help to shed further light on this issue.\(^ {39}\)

In summary, early prospective studies in both healthy individuals and first-time stroke patients have found an association between elevated plasma CRP levels and future risk of stroke. More prospective studies are needed to further examine the association of CRP with stroke, the optimal time of CRP measurement after stroke, and the effect of drugs aimed at lowering plasma CRP levels on future risk of stroke. The more firmly established association between elevated CRP and risk for acute coronary events has led the American Heart Association and Centers for Disease Control to issue joint guidelines regarding the utility of CRP measurement in the assessment of primary and secondary risk of cardiovascular disease-related events. Such testing is favored in patients judged at intermediate risk for coronary heart disease or in those with stable coronary disease or acute coronary syndromes, although it may be used at the physician's discretion as part of global coronary risk assessment among healthy adults without known cardiovascular disease.\(^ {40}\) Based on these guidelines, it appears worthwhile to consider CRP measurement in patients at risk for stroke or with a history of cerebrovascular disease to assist in risk assessment for future cerebrovascular or cardiovascular events. The recommended high-sensitivity CRP cut-off points for cardiovascular risk assessment are as follows: less than 1.0 mg/L for low risk, 1.0 to 3.0 mg/L for average risk, and more than 3.0 mg/L for high risk for future cardiovascular events.\(^ {41}\)

**HOMOCYSTEINE**

Hyperhomocysteinemia has been implicated as a risk factor for venous thrombosis and coronary artery disease, as well as for stroke. Homocysteine is an amino acid derived from methionine, which can be converted into cysteine or metabolized back into methionine. Metabolic pathways involving homocysteine require vitamin B\(_6\) (pyridoxine), vitamin B\(_{12}\) (cobalamin), and folic acid to function normally (Figure 1). Elevated serum homocysteine therefore may be genetic, due to polymorphisms or mutations in enzymes involved in these metabolic pathways, or acquired, due to dietary deficiencies of vitamin B\(_6\), vitamin B\(_{12}\), or folic acid.\(^ {42}\) Other acquired causes of elevated homocysteine include renal dysfunction, hypothyroidism, malignancy, or certain medications, such as methotrexate, theophylline, or phenytoin.

Most prospective studies in general or of healthy populations, including men and women of various age groups and ethnic backgrounds, show an association between elevated serum homocysteine and subsequent risk of stroke;\(^ {43}-45\) and a recent meta-analysis concluded that elevated homocysteine is a risk factor for cerebrovascular disease.\(^ {46}\) Other prospective studies have shown that the effect may be limited to certain individuals. Fallon et al.\(^ {47}\) demonstrated an association between elevated homocysteine and increased stroke risk that was limited to men younger than 65 years, whereas Stehouwer et al.\(^ {48}\) showed that an association with increased stroke mortality was found only in normotensive individuals. A triethnic cohort study of 3298 subjects showed that elevated homocysteine was a stronger predictor of ischemic stroke among whites and Hispanics compared with blacks.\(^ {44}\) Other prospective studies have shown that elevated homocysteine may be associated with neurologic disease other than stroke. Data from the Framingham Study of 1092 subjects without dementia at baseline reveal that elevated homocysteine was associated with an increased risk for dementia and Alzheimer's disease during an 8-year follow-up period.\(^ {49}\)

Other studies in general or healthy populations have shown more conflicting results. Two prospective studies of healthy middle-aged and elderly individuals without stroke or other atherosclerotic disease at baseline showed no association between elevated homocysteine levels and increased risk of stroke.\(^ {50,51}\)

Among patients with a history of stroke, 1 longitudinal study of 1039 acute stroke patients showed that serum homocysteine levels measured within 24 hours of admission were significantly higher in patients who experienced a recurrent stroke than in patients without recurrence during a 15-month follow-up.\(^ {52}\) Among 78 patients with a history of stroke or TIA, elevated homocysteine levels were significantly correlated with the progression of aortic atheroma as measured by transesophageal echocardiography during a period of 9 months.\(^ {53}\) Among patients with existing stable coronary heart disease, a prospective, nested, case-control
showed a similar trend, with homocysteine levels increasing from 8.5 μmol/L at a median of 100 days post stroke. Howard et al58 examined the change in homocysteine levels only within the acute setting after stroke and also showed an increasing trend, from 11.3 μmol/L at 1 day post stroke to 13.7 μmol/L after 14 days. Because prestroke homocysteine levels were unknown in any of these studies, it is not clear whether homocysteine levels are temporarily decreased during the acute setting after a stroke or if homocysteine levels become elevated as a consequence of stroke.

A particular mutation in the methylene tetrahydrofolate reductase (MTHFR) gene encoding one of the enzymes of the homocysteine metabolic pathway is relatively common in the general population. The mutation, which involves a C to T transition at nucleotide 677, is asymptomatic in the heterozygous form. Most,69–73 but not all,64,66 studies of homozygous form, MTHFR 677TT, in adults have not shown an association with an increased risk of stroke, including 2 negative studies in young adults 15 to 45 years66,67 and 1 study with borderline significant results in young adults younger than 50 years. In a retrospective study of young women aged 20 to 49 years, MTHFR 677TT was not associated with stroke in women not taking oral contraceptives, but the risk increased to 5.4-fold in homozygotes using oral contraceptives as compared with study participants lacking both risk factors.71 In contrast, a meta-analysis of MTHFR 677TT revealed a modest but statistically significant association with ischemic stroke (odds ratio, 1.26).72 Results of retrospective studies of MTHFR 677TT in children have been conflicting, with both positive73–75 and negative76–79 results.

Studies of homocysteine with cerebral venous thrombosis are limited. In a case-control study involving 121 patients and 242 controls, Martinelli et al80 found a significant association between elevated homocysteine and cerebral venous thrombosis (odds ratio, 4.2), whereas in another study of similar design involving 26 patients and 100 controls, Boncoraglio et al81 found a comparable odds ratio of 4.18.

The utility of lowering the homocysteine level with vitamin therapy to decrease the risk of subsequent stroke is under investigation.82,83 Preliminary evidence was provided in a series of 38 patients with atherosclerosis and elevated serum homocysteine in whom progression of carotid artery atherosclerosis was slowed by lowering homocysteine with folic acid, vitamin B, and vitamin B, supplementation.84 Some evidence from prospective observational data also suggests that increased folate and vitamin intake is associated with a lower risk of future stroke. In a study of 43 732 adult men free of cardiovascular disease at baseline, higher dietary folate intake was associated with a significantly lower risk of ischemic stroke after 14 years of follow-up (adjusted relative risk, 0.71).85 Dietary intake of vitamin B, but not B, was also inversely associated with risk of ischemic stroke.86 However, in a similarly designed study of 83 272 healthy adult women followed for 18 years, no appreciable association between increased folate intake and stroke incidence was observed (adjusted relative risk, 1.01).87 In the Vitamin
Intervention for Stroke Prevention (VISP) trial, a large, double-blind, randomized control trial of 3680 adults with non-disabling cerebral infarction and baseline homocysteine level above the 25th percentile of the North American stroke population, subjects were assigned to receive either a high-dose or a low-dose formulation of folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>. The study found that the greater mean reduction of plasma homocysteine levels seen in the group randomized to the high-dose formulation did not result in a decreased recurrence of cerebral infarction or other outcomes (coronary heart disease events and death) during 2 years of follow-up. The authors noted, despite these negative findings, a consistent association of lower homocysteine levels and lower vascular risk and suggested that trials in populations with higher baseline homocysteine levels with longer follow-up may be warranted.

In summary, most but not all of the prospective studies in healthy or general populations have found an association between homocysteine and future risk of stroke. There are no prospective data linking the MTHFR 677TT mutation with increased risk of stroke. Studies investigating the role of lowering homocysteine levels to help reduce the risk of future cerebrovascular disease are ongoing. However, because homocysteine levels are easily reduced by supplementation with B vitamins (vitamins B<sub>6</sub>, B<sub>12</sub>, and folic acid), it appears worthwhile to consider measuring homocysteine levels in patients with stroke, high risk for stroke, existing atherosclerosis, or cerebral venous thrombosis.

**LIPOPROTEIN(a)**

Lipoprotein(a) is a low-density lipoprotein cholesterol particle with an additional protein covalently attached called apolipoprotein(a). The majority of studies involving Lp(a) and stroke have not been prospective. Prospective studies in healthy populations have generated conflicting findings. Boslom et al. found that elevated Lp(a) was associated with future risk of stroke or TIA, whereas Nguyen et al. found a similar, but inconsistent, association. In a prospective study of 5888 older adults (>65 years) who were healthy at baseline, men, but not women, in the highest Lp(a) quintile had 3 times the risk of future stroke as compared with men in the lowest Lp(a) quintile. Adjustment for other factors, including age, sex, and serum lipids, had little effect on the overall increased risk. In another prospective study of healthy, elderly individuals, Lp(a) was higher in those who later developed stroke (with or without dementia), but the trend did not reach statistical significance (<i>P = .06</i>). Other prospective studies have not found any association between elevated Lp(a) and future risk of stroke in healthy individuals.

In a large prospective cohort study of 5732 elderly individuals aged 70 to 82 years, some of whom had a history of vascular disease including stroke, Gaw and colleagues reported that subjects with higher plasma Lp(a) levels had a greater risk of future cardiovascular and cerebrovascular events after adjustment for baseline risk factors (<i>P = .03</i>). However, when the composite primary endpoint was split into its coronary and cerebrovascular components and each was analyzed separately, this association lost statistical significance. In a Scandinavian study of 2372 subjects, some with a history of cardiovascular disease, plasma Lp(a) had only a borderline significant association with future stroke and coronary events.

In studies limited to individuals with a history of stroke or TIA, 1 prospective study of 151 adult patients found no association between elevated baseline Lp(a) and subsequent risk of recurrent stroke, myocardial infarction, or death. However, in 301 children aged 6 months to 18 years with a history of ischemic stroke, the risk of a second stroke was significantly increased in patients with elevated Lp(a).

Testing for Lp(a) is complicated by the fact that this marker is also an acute phase reactant and can become elevated in acute phase settings. Studies may be hindered if an observed Lp(a) elevation is the result of thrombosis-induced acute phase reaction. However, 1 study showed that there was no significant change in Lp(a) during the first 4 weeks following a stroke. In addition, certain assays for Lp(a) that detect a repeat domain in the Lp(a) molecule called kringle 4 can overestimate the level of Lp(a) if the patient has large apolipoprotein (a) isoforms and underestimate the level if the patient has small apolipoprotein (a) isoforms.

In summary, prospective studies investigating the relationship between Lp(a) and stroke or TIA have been conflicting, and it is therefore difficult to make a recommendation about routine testing for Lp(a) in stroke patients. Six of the 11 prospective studies described previously found an association (and a seventh study among the 11 showed a nonsignificant trend in favor of an association). On that basis, and because Lp(a) can be reduced by niacin therapy, measurement of Lp(a) can be considered for patients with stroke or TIA. However, further study is needed to determine if treatment to reduce levels of Lp(a) with niacin or related compounds results in a reduction of subsequent risk of thrombosis.

**PROTHROMBIN G20210A MUTATION**

The G20210A point mutation in factor II or prothrombin results in increased plasma levels of prothrombin and an increased risk of venous thrombosis in its heterozygous form. Homozygous prothrombin G20210A is, as expected, less common than the heterozygous form but is believed to have an even greater risk for venous thrombosis. Very few studies investigating the relationship between prothrombin G20210A and ischemic arterial stroke or cerebral venous thrombosis have been prospective. The results of the prospective, nested, case-control Physician's Health Study involving 259 cases of stroke among 14,916 men yielded no association between prothrombin G20210A and stroke. A prospective cohort study found that the annual incidence of arterial cardiovascular events (including stroke, TIA, and myocardial infarction) was not increased by prothrombin G20210A. An ongoing prospective study of 820 patients with recent stroke or TIA is evaluating the association of prothrombin G20210A with risk of subsequent vascular events in this population.

Similarly, several retrospective case-control studies have not found an association between prothrombin G20210A and stroke or TIA, including studies involving younger adults younger than 45 to 50 years. However, in 1 study involving 72 patients with stroke younger than 50 years who lacked other risk factors, De Stefano et al. found that prothrombin G20210A was significantly more common in cases than in controls. In another study involving 49 patients younger than 50 years with cryptogenic stroke, Aznar et al. also found an as-
Figure 2. Role of natural anticoagulants in the coagulation cascade. Activated protein C inhibits coagulation by degrading activated factors V and VIII, with protein S participating as a cofactor in this degradation. Individuals with activated protein C resistance have a mutation in factor V such that it is resistant to degradation by activated protein C. Antithrombin is a natural inhibitor of thrombin, as well as of activated factors X, IX, XI, and XII. A hereditary deficiency of any of the 3 natural anticoagulants (protein C, protein S, or antithrombin) results in a hypercoagulable state. Ca\(^{2+}\) indicates calcium; HMWK, high-molecular-weight kininogen; PL, phospholipid; PK, prekallikrein; and TF, tissue factor.

Association with prothrombin G20210A. A meta-analysis of published case-control and cohort studies revealed only a modest association between prothrombin G20210A and risk for myocardial infarction or ischemic stroke (odds ratio 1.32), with slightly stronger associations in women and younger patients (<55 years).\(^ {108}\) A more recent meta-analysis of 3028 cases from 19 published case-control studies evaluating prothrombin G20210A and the risk of ischemic stroke revealed a similar degree of association (odds ratio, 1.44; statistically significant).\(^ {109}\) Pediatric populations, including neonates, infants, and children, have also been studied. In a small prospective study of 44 children with arterial ischemic stroke, Bonduel and colleagues\(^ {110}\) found no cases of prothrombin G20210A. Several retrospective case-control studies of infants and children suggest that prothrombin G20210A may be a risk factor for cerebral infarction in these populations.\(^ {73,76,111}\) Although other such studies and 1 meta-analysis have been negative for any association.\(^ {77,78,112}\) In a study of 91 neonates with stroke, prothrombin G20210A was more common in cases than in controls, but this difference was not statistically significant.\(^ {79}\) In a case series of 24 neonates with cerebral infarction, no cases of prothrombin G20210A were identified.\(^ {113}\)

Retrospective studies of cerebral venous thrombosis have yielded results supporting an association with prothrombin G20210A. The mutation was more common in 40 patients with cerebral vein thrombosis as compared with controls.\(^ {114}\) In addition, this study showed that the risk for cerebral vein thrombosis was increased among women with prothrombin G20210A who also took oral contraceptives,\(^ {114}\) suggestive of a synergistic effect. Similar associations between prothrombin G20210A and cerebral vein thrombosis have been found in several other retrospective case-control studies.\(^ {66,101,115-118}\) In contrast, a small prospective study of 23 children with cerebral venous thrombosis showed no association with prothrombin G20210A.\(^ {110}\)

In summary, prothrombin G20210A may be more prevalent among pediatric patients with stroke as compared with controls, although the data are conflicting and large-scale prospective studies are lacking. Retrospective, case-control studies suggest that prothrombin G20210A is more prevalent among patients with cerebral vein thrombosis versus controls. In light of available data, routine testing for prothrombin G20210A is generally not informative in adult patients with ischemic arterial stroke, but testing for the mutation is a consideration in certain clinical circumstances, such as pediatric patients with stroke and patients with cerebral venous thrombosis.

**FACTOR V LEIDEN (ACTIVATED PROTEIN C RESISTANCE)**

Activated protein C resistance is the most common known hereditary predisposition to venous thrombosis.\(^ {2}\) Activated protein C acts as a natural anticoagulant by degrading activated factors V and VIII via proteolytic cleavage at specific arginine residues (Figure 2). Individuals with activated protein C resistance have a mutation in factor V such that it is resistant to degradation by activated protein C. Greater than 95% of cases of activated protein C resistance are due to a specific point mutation in factor V, the factor V Leiden mutation, at one of the arginine cleavage sites.\(^ {2}\)

The association between the factor V Leiden mutation and ischemic arterial stroke has been investigated prospectively in only a few studies. In the prospective, nested cases-control Physician’s Health Study of 209 stroke cases among 14,916 initially healthy men, factor V Leiden was not significantly associated with an increased risk of stroke.\(^ {119}\) The Cardiovascular Health Study of 216 stroke or TIA cases among 5201 initially healthy men and women...
older than 65 years showed that factor V Leiden was not a risk factor for future stroke or TIA during a 3.4-year follow-up period. In the Stroke Prevention in Atrial Fibrillation III study of 752 adult patients with atrial fibrillation on aspirin, factor V Leiden was not associated with an increased risk of ischemic stroke or systemic embolization. Factor V Leiden had no association with stroke in the Copenhagen City Heart Study of 410 stroke cases among 8835 initially healthy men and women with 21-year follow-up. These authors also performed a meta-analysis of 8 studies and found no association between factor V Leiden and stroke in adults. The risk conferred by factor V Leiden on the occurrence of new vascular events in patients with a history of stroke or TIA has not been prospectively studied; the ongoing Polymorphisms and Risk of Ischemic Stroke (POLARIS) study is attempting to answer this question.

Several retrospective case-control studies have also failed to demonstrate a significant association between factor V Leiden and stroke, mild or moderate stroke, stroke or TIA, and TIA or minor stroke. Negative retrospective studies have shown consistent findings in both older (>50 years) and younger (approximately 15–50 years) populations. In 3 of the negative studies cited previously, a trend toward an association was seen but was not found to be statistically significant. Nabavi et al. cited previously found a significant association between factor V Leiden and the subgroup of patients with cryptogenic stroke, whereas Aznar et al. did not. Another study involving women aged 20 to 49 years revealed an 11.2-fold increased risk of ischemic stroke in factor V Leiden mutation carriers who were also using oral contraceptives but no association in carriers not using oral contraceptives. The results suggested a possible synergistic effect between factor V Leiden and oral contraceptive use.

In contrast to the majority of findings, 1 retrospective study involving individuals 50 years of age or younger did demonstrate an association between factor V Leiden and stroke, whereas 2 meta-analyses revealed modest associations between factor V Leiden and ischemic stroke (odds ratio 1.33, statistically significant) and myocardial infarction or ischemic stroke (odds ratio 1.21, not significant), with a slightly stronger association in women and younger patients (<55 years) seen in the latter study. A retrospective study investigating genetic mutations in the setting of clinical risk factors found that the factor V Leiden mutation increased the risk of stroke in patients with hypertension or diabetes mellitus.

Among studies of pediatric populations, a small prospective study of 44 children with arterial ischemic stroke showed no increased prevalence of factor V Leiden in cases versus controls. In contrast, a meta-analysis and several retrospective, case-control studies of infants and children have found that factor V Leiden was significantly associated with stroke. Other studies have been negative, although nonsignificant trends have been found. A retrospective, case-control study of 91 neonates with stroke also found an association between factor V Leiden and stroke. Lastly, in a case series of 24 neonates with cerebral infarction, 5 patients (21%) had factor V Leiden. Follow-up of these patients at age 2 or older revealed that all 5 patients (100%) with factor V Leiden had hemiplegia, whereas only 21% of patients without factor V Leiden had hemiplegia.

Several retrospective studies have demonstrated that factor V Leiden is more common in patients with cerebral venous thrombosis as compared with controls. A nonsignificant trend was seen in 3 additional studies. In contrast, 1 small prospective study of 23 children with cerebral venous thrombosis showed no increased prevalence of factor V Leiden in cases versus controls.

In summary, the association between factor V Leiden and stroke appears similar to that of prothrombin G20210A and stroke. Factor V Leiden may be more prevalent among pediatric stroke patients as compared with controls, although prospective studies are lacking in this group. Retrospective, case-control studies also suggest an increased prevalence of factor V Leiden among patients with cerebral venous thrombosis versus control patients. Testing for the factor V Leiden mutation is therefore a consideration in pediatric stroke patients or in patients with cerebral venous thrombosis; however, routine testing of adult patients with ischemic arterial stroke is generally not informative.

### PROTEIN C, PROTEIN S, OR ANTITHROMBIN DEFICIENCIES

Protein C, protein S, and antithrombin are natural anticoagulants. As described previously, protein C, after becoming activated, inhibits coagulation by degrading activated factors V and VIII. Protein S participates as a cofactor in this degradation. Antithrombin is a natural inhibitor of thrombin (activated factor II), as well as an inhibitor of activated factors X, IX, XI, XII, and kallikrein. Its activity is greatly enhanced by interaction with glycosaminoglycans, including heparan sulfate on endothelial cell surfaces and exogenously administered heparin. Deficiency of any of these 3 natural anticoagulants results in a hypercoagulable state (Figure 2).

Prospective studies examining the risk of stroke in these deficient states have provided conflicting data. Folsom et al. found that lower baseline protein C levels, but not antithrombin levels, had a borderline significant association with the risk of stroke during a 6- to 9-year follow-up. It was not known in this study which patients, if any, had hereditary protein C or antithrombin deficiency. Knuiman et al. found that lower baseline protein C levels were significantly associated with cerebral infarctions as identified by magnetic resonance imaging performed during a 6-year follow-up; however, baseline magnetic resonance imagings were not performed for comparison. In a prospective study of 301 children aged 6 months to 18 years with a history of ischemic stroke, Strater and colleagues confirmed the familial nature of the deficiency by repeat testing and testing of family members and found that the risk of a subsequent stroke was significantly increased in patients with familial protein C deficiency but not for deficiencies of protein S or antithrombin. In contrast to these findings, Finazzi et al. found no cases of stroke occurrence among 36 patients with hereditary protein C deficiency, 36 patients with hereditary protein S deficiency, and 9 patients with hereditary antithrombin deficiency during 160 patient-years of follow-up.

Other studies examining the risk of stroke in deficiencies of protein C, protein S, and antithrombin have been retrospective. In a Japanese study of 26800 cardiovascular inpatients, the age of stroke onset among patients with hereditary protein C deficiency was significantly younger as compared with patients with normal protein C status.
A retrospective study of 150 families revealed that 0% of antithrombin, 1.6% of protein C, and 4.8% of protein S deficient subjects experienced stroke, as compared with 0.6% of relatives without any of the 3 deficiencies. In this study, the overall rate of arterial thrombotic events (either stroke or myocardial infarction) was not significantly different between deficient subjects and their non-deficient relatives. In a meta-analysis of 18 case-control studies of children with first-time arterial ischemic stroke, protein C deficiency, but not protein S or antithrombin deficiency, showed a significant association with an increased risk of stroke (odds ratio, 6.49).

Such data must be interpreted with caution, as testing for hereditary deficiencies of protein C, protein S, or antithrombin is complicated by the fact that acquired decreases in these anticoagulants are far more common than hereditary deficiencies. Protein S is decreased by acute events that often occur as a result of a thrombotic event. All 3 proteins are consumed by thrombosis, surgery, or disseminated intravascular coagulation, and all 3 may be decreased in liver disease, due to decreased hepatic synthesis. Heparin can decrease antithrombin, whereas warfarin and vitamin K deficiency decrease both protein C and protein S. Protein S, and sometimes antithrombin, are decreased by pregnancy or estrogen use. Acquired, transient decreases in the levels of protein C, protein S, and antithrombin that disappear on retesting are commonly encountered in stroke patients. In a study that confirmed abnormal test results by repeating the assay after 3 to 6 months while off anticoagulants for at least 10 days and that confirmed the hereditary nature of the deficiency with testing of family members, 2 out of 30 children with stroke (6.7%) had hereditary protein S deficiency, 1 (3.3%) of 30 had hereditary protein C deficiency, and no child had antithrombin deficiency. In other studies, no deficiencies of protein C, protein S, or antithrombin were identified among 202 individuals aged 3 to 50 years with stroke, or 37 children with stroke, or 24 neonates with stroke. Several studies have found a possible association between protein C deficiency and stroke in children, but exclusion of acquired etiologies confirms a family testing, and repeat testing were generally not performed. Cases of protein S and antithrombin deficiency were not identified in these studies.

In summary, the prevalence of hereditary deficiencies of protein C, protein S, or antithrombin are uncommon in general populations, as well as in persons with stroke, although there is some evidence to suggest an association with stroke for protein C. However, due to their low prevalence and the complexities of distinguishing acquired from hereditary deficiencies, it is difficult to ascertain the true risk of stroke when baseline levels of these anticoagulants are decreased. It may be worthwhile considering these assays in certain clinical situations, such as in young patients with stroke and in patients with a personal or family history of thrombosis or paradoxical emboli. These tests are also appropriate for patients with cerebral venous thrombosis, extrapolating from their well-established association with venous thrombosis.

**HEPARIN-INDUCED THROMBOCYTOPENIA**

Heparin-induced thrombocytopenia is a consideration in any patient who experiences ischemic stroke or cerebral venous thrombosis while exposed to heparin, within 30 days of heparin exposure, or in any patient with a greater than 50% decline in platelet count from baseline while on heparin. A detailed discussion of heparin-induced thrombocytopenia is beyond the scope of this review.

**CONCLUSION**

In summary, a great deal of data is accumulating regarding the utility of various hypercoagulability risk factors in evaluation of the risk of initial or recurrent stroke. This review has attempted to summarize available data, with an emphasis on prospective studies, as well as to review current data regarding treatment of various risk factors.

The importance of testing for various risk factors is underscored by the fact that certain risk factors, such as homocysteine, CRP, and Lp(a), may be treatable by vitamins, lipid-lowering drugs, or niacin, respectively. In addition, some test results could influence other aspects of management. For example, lupus anticoagulants can interfere with heparin monitoring if heparin is administered, resulting in heparin underdosing if the presence of the lupus anticoagulant is not known. A positive test result might allow the patient to be monitored for a hypercoagulable condition that may have other clinical manifestations, such as an increased risk for venous thrombosis with elevated homocysteine, antiphospholipid antibodies, or other conditions or an increased risk for myocardial infarction with elevated homocysteine, CRP, Lp(a), or antiphospholipid antibodies.

For pediatric stroke patients, prospective data are limited regarding antiphospholipid antibodies, CRP, homocysteine, and Lp(a). Therefore, the use of these tests in pediatric stroke patients is based largely on extrapolation from adult prospective studies. Prospective data are limited for pediatric as well as adult patients regarding prothrombin G20210A, factor V Leiden, and deficiencies of protein C, protein S, and antithrombin III. The use of these tests in pediatric stroke patients is based largely on retrospective studies of stroke in pediatric populations.

Certain tests, such as CRP, homocysteine, antiphospholipid antibodies, and Lp(a), may be useful in patients with a history of stroke or at high risk for stroke, as evidenced by prospective data, although prospective data are somewhat conflicting for antiphospholipid antibodies and Lp(a). Other tests, such as prothrombin G20210A, factor V Leiden, protein C, protein S, and antithrombin, are not recommended for routine testing but may be useful in pediatric patients with stroke or in patients with cerebral vein thrombosis. Finally, certain tests lacking prospective data, such as MTHFR 677TT, are not recommended for routine testing in stroke.

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