High-Sensitivity C-Reactive Protein and Lipoprotein-Associated Phospholipase A\textsubscript{2} Stability Before and After Stroke and Myocardial Infarction

Mitchell S.V. Elkind, MD, MS; Vladimir Leon, BS; Yeseon P. Moon, MS; Myunghee C. Paik, PhD; Ralph L. Sacco, MD, MS

Background and Purpose—High-sensitivity C-reactive protein (hsCRP) and lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}) are hypothesized to be biomarkers of systemic inflammation and risk of myocardial infarction (MI) and stroke. Little is known, however, about the stability of these markers over time, and in particular, about the effects of acute vascular events on these marker levels.

Methods—Serum samples were collected at 4 annual intervals in 52 stroke-free participants from the Northern Manhattan Study (NOMAS) and assayed for hsCRP and Lp-PLA\textsubscript{2} mass and activity levels using standard techniques. Log transformation of levels was performed as needed to stabilize the variance. Stability of marker levels over time was assessed using random effects models unadjusted and adjusted for demographics and other risk factors. In addition, samples from 37 initially stroke-free participants with stroke (n=17) or MI (n=20) were available for measurement before and after the vascular event (median 5 days, range 2 to 40 days). Levels before and after events were compared using nonparametric tests.

Results—HsCRP and Lp-PLA\textsubscript{2} activity levels were stable over time, whereas Lp-PLA\textsubscript{2} mass levels decreased on average 5\% per year (P=0.0015). Using accepted thresholds to define risk categories of Lp-PLA\textsubscript{2} mass, there was no significant change over time. HsCRP increased after stroke (from median 2.2 mg/L prestroke to 6.5 mg/L poststroke; P=0.0067) and MI (from median 2.5 mg/L pre-MI to 13.5 mg/L post-MI; P<0.0001). Lp-PLA\textsubscript{2} mass and activity levels both decreased significantly after stroke and MI (for Lp-PLA\textsubscript{2} mass, from median 210.0 ng/mL to 169.4 ng/mL poststroke, P=0.0348, and from median 233.0 ng/mL to 153.9 post-MI, P<0.0001).

Conclusion—Lp-PLA\textsubscript{2} mass levels decrease modestly, whereas hsCRP and Lp-PLA\textsubscript{2} activity appear stable over time. Acutely after stroke and MI, hsCRP increases whereas Lp-PLA\textsubscript{2} mass and activity levels decrease. These changes imply that measurements made soon after stroke and MI are not reflective of prestroke levels and may be less reliable for long-term risk stratification. (Stroke. 2009;40:3233-3237.)

Key Words: C-reactive protein ■ biomarker ■ inflammation ■ ischemic stroke ■ myocardial infarction

Systemic inflammatory biomarkers, including high-sensitivity C-reactive protein (hsCRP) and lipoprotein-associated phospholipase A\textsubscript{2} (Lp-PLA\textsubscript{2}), predict risk of first and recurrent myocardial infarction (MI) and, possibly, stroke.\textsuperscript{1,2,3} Some studies provide evidence that hsCRP levels in disease-free individuals may fluctuate from one visit to the next, suggesting limited utility of this measure.\textsuperscript{4,5} There is relatively little data available, however, about hsCRP stability in multi-ethnic population-based cohorts and even less about stability of Lp-PLA\textsubscript{2} over time.

Measurement of inflammatory biomarkers after ischemic events for the purpose of secondary risk prognostication may also be limited by the impact of the event itself on the biomarker. In the Northern Manhattan Study (NOMAS), for example, hsCRP levels measured acutely after stroke were associated with stroke severity and were higher among patients with stroke than among stroke-free participants from the same population.\textsuperscript{6,7} HsCRP was associated with mortality but not recurrence of stroke or other vascular events. Lp-PLA\textsubscript{2} levels were not associated with stroke severity, however, and predicted subsequent vascular events, including recurrent stroke. It remains largely unexplored, however, whether levels of these biomarkers change when measured before and after an event in the same individual. Because we have early access to many of the participants in our prospective cohort study who are hospitalized with acute MI and stroke, we were able to compare levels of these biomarkers before and soon after vascular events.
We hypothesized that (1) levels of these biomarkers would remain stable on repeated measurement in event-free individuals over the course of several years, and (2) levels would change as a result of acute vascular events.

Methods
Selection and Evaluation of Participants
NOMAS includes a population-based prospective cohort study in a multi-ethnic urban population. Methods of participant recruitment, assessment, and follow-up have been described previously. Briefly, stroke-free participants were identified by random digit dialing. Participants were eligible if they had no history of stroke, were ≥40 years of age, and if they resided in northern Manhattan for ≥3 months. Baseline assessment included medical history ascertained using questions adapted from the Behavioral Risk Factor Surveillance System from the Centers for Disease Control and Prevention,9 neurological examination, and questionnaires regarding diet and alcohol consumption. Hypertension was defined by history of hypertension or use of antihypertensive medications, and diabetes by fasting blood glucose level ≥126 mg/dL or self-reported history of insulin or oral hyperglycemic use.8

The cohort was followed with annual telephone interviews and local hospital surveillance for stroke, myocardial infarction, and death. A 10% subsample returned for annual in-person visits. Serum samples were collected at 4 annual intervals in 52 participants and assayed for hsCRP and LpPLA2 mass and activity levels. In addition, samples from 37 initially stroke-free participants with stroke (n=17) or MI (n=20) were available for measurement before and after the vascular event (median 5 days, interquartile range 4 to 8 days, range 2 to 40 days).

The study was approved by the Columbia University Medical Center (CUMC) Institutional Review Board, and all patients provided informed consent.

Biomarker Assays
Blood samples were collected at the time of clinic visit or hospitalization in 5 mL serum separator tubes by a trained phlebotomist, centrifuged at 3000g for 15 minutes, and then aliquoted into 2 mL Eppendorf tubes. Samples were stored at −80°C until assays were run. Serum samples were assayed for hsCRP using an enzyme-linked immunoassay (BioCheck), and Lp-PLA2 mass using a microplate-based ELISA (PLAC assay, diaDexus Inc) as previously described. LpPLA2 activity was measured with a colorimetric method (diaDexus Inc). Assays were run at a central laboratory at diaDexus Inc. Laboratory personnel were blinded to all patient clinical data and outcomes.

Statistical Analysis
Means and standard deviations of hsCRP, Lp-PLA2 mass, and Lp-PLA2 activity were calculated. Levels of markers were log-transformed before analysis as needed to stabilize the variance. Stability of marker levels over time was assessed using random effects models before and after adjusting for demographics and other vascular risk factors, including level of low-density lipoprotein (LDL) and leukocyte count. Terms for interactions between use of lipid-lowering medication and change in marker levels over time were also added to account for the possible effect of use of these medications on marker levels. Marker levels before and after stroke or MI were compared using nonparametric tests (signed Wilcoxon). Type I error was set at 0.05. Statistical analysis was conducted using SAS Version 8.2 (SAS Institute).

Results
Serial Measurements of Biomarkers Over Time Among Those Without Events
Table 1 shows the baseline demographic and vascular disease risk factor profile of the 52 subjects with 4 annual repeated measurements available for this analysis. Figure 1 shows box and whisker plots for crude measures of the biomarkers over annual intervals. Table 2 shows results for log-transformed values over time, both unadjusted and adjusted for demographics and risk factors. There was no evidence of a time trend in levels of hsCRP and Lp-PLA2 activity. Lp-PLA2 mass levels showed a modest decrease over time (5% per year, P=0.0041 after adjusting for other risk factors). There was marginal evidence of an interaction with the use of lipid-lowering medications during follow-up (P=0.0681), indicating a larger reduction in Lp-PLA2 mass among those on these medications. For those on these medications, there was a significant reduction in Lp-PLA2 mass over time (8% per year, P=0.0009), whereas among those not on these medications there was no significant reduction (3% per year, P=0.0655). No interaction with medication was present for hsCRP.

Because Lp-PLA2 mass levels have formerly been dichotomized into high (>235 ng/mL) and low-to-moderate (<235 ng/mL) risk categories,13 additional analyses were undertaken to determine whether risk categories changed during follow-up. There was no significant change in categories over time (P=0.23). Using a more recent dichotomization (high risk ≥200 ng/mL and low-to-moderate risk <200 ng/mL),14 there was evidence of annual decline in the proportion of those with Lp-PLA2 mass ≥200 ng/mL (adjusted OR=0.70, P=0.01) The change in categories over time was significant among those taking lipid lowering medications (adjusted OR=0.59, P=0.0189), but not for those not on medications (adjusted OR=0.77, P=0.0694).

Differences in Biomarker Levels Before and After Acute Events
Figure 2 shows the levels of hsCRP before and after acute vascular events, both separately for stroke and MI and as a combined group. The mean time between the preevent and the postevent measurement was 3.0 ± SD 2.1 years (median 3.3 years).

Table 1. Baseline Characteristics of Stroke-Free Participants

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<th>n</th>
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<tbody>
<tr>
<td>Age, y (mean±SD)</td>
<td>70.5±9.9</td>
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<tr>
<td>Men, n (%)</td>
<td>16 (30.8)</td>
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<tr>
<td>Race-ethnicity, n (%)</td>
<td></td>
<td></td>
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<tr>
<td>Non-Hispanic white</td>
<td>23 (44.2)</td>
<td></td>
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<tr>
<td>Non-Hispanic black</td>
<td>14 (26.9)</td>
<td></td>
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<tr>
<td>Hispanic</td>
<td>15 (28.9)</td>
<td></td>
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<tr>
<td>Hypertension, n (%)</td>
<td>42 (80.8)</td>
<td></td>
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<tr>
<td>Current smoking, n (%)</td>
<td>8 (15.4)</td>
<td></td>
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<tr>
<td>Diabetes mellitus, n (%)</td>
<td>7 (13.5)</td>
<td></td>
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<tr>
<td>Coronary disease, n (%)</td>
<td>14 (26.9)</td>
<td></td>
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<tr>
<td>hsCRP, mg/L (median)</td>
<td>1.09</td>
<td></td>
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<tr>
<td>Lp-PLA2 levels (mean±SD)</td>
<td></td>
<td></td>
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<tr>
<td>Mass, ng/mL</td>
<td>268.8±99.8</td>
<td></td>
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<tr>
<td>Activity, nmol/min/mL</td>
<td>149.5±29.8</td>
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HsCRP increased after stroke from a median of 2.2 mg/L pre-stroke to 6.5 mg/L post-stroke (P=0.0067). Levels increased after MI even more, from a median of 2.5 mg/L pre-MI to 13.5 mg/L post-MI (P<0.0001). Lp-PLA2 mass and activity levels both decreased significantly after stroke and MI. For Lp-PLA2 mass, levels decreased from a median of 210.0 ng/mL to 169.4 ng/mL post-stroke (P=0.05 0.36) and from a median of 233.0 ng/mL to 153.9 post-MI (P=0.0041). Lp-PLA2 activity decreased from 145.9 to 129.2 nmol/min/mL post-stroke (P=0.0348) and from 144.0 to 99.2 post-MI (P<0.0001).

Discussion

Inflammation is increasingly recognized to play an important role in atherosclerosis and stroke.1,15 Macrophages, cytokines, and leukocyte adhesion molecules contribute to vascular injury, endothelial dysfunction, plaque formation, plaque rupture, and coagulopathy.16 Because of this, serum levels of inflammatory biomarkers such as hsCRP, an acute phase protein, have been used as nonspecific measures of vascular inflammation. Lp-PLA2 is an enzyme derived from leukocytes, particularly macrophages, that is involved in metabolism of LDL to the proinflammatory mediators lysophosphatidylcholine and oxidized fatty acids.3,11 Lysophosphatidylcholine increases expression of vascular adhesion molecules, upregulates cytokines and CD40 ligand, and stimulates macrophage proliferation. Our findings provide evidence that hsCRP and Lp-PLA2 activity levels are stable when repeated annually. Our findings are similar to findings of stability for hsCRP from other populations.17,18 In one study among patients with stable ischemic heart disease,4 hsCRP levels changed across sampling times, and these changes were associated with changes in risk categories.19 This study population differs from our own, however, in including only patients with coronary disease, using variable numbers of measurements per patient, and using measurements at time points from 15 days to 6 years. In our study, moreover, we modeled change while adjusting for other risk factors.

Similar data on stability of Lp-PLA2 are lacking, however. We found evidence of a modest decrement in Lp-PLA2 mass levels, though these changes were not sufficient to change risk categories in most patients. In individuals on lipid lowering therapies, however, levels decreased more dramatically and there was a change in risk category levels, depending on threshold used. This finding is consistent with studies that demonstrated effects of lipid lowering therapies in lowering Lp-PLA2.20,21 Though further studies are warranted to determine the precise role of these biomarkers in clinical management, the present results indicate that levels can probably be considered a stable measurement in individuals free of intercurrent events.
Our study provides evidence, moreover, that biomarker levels change in the acute phase of acute events, such as stroke and MI. Our study is unique in having biomarker levels available both before and after events in the same individuals. Of note, biomarkers changed in opposite directions. HsCRP levels increased after both stroke and MI, whereas both Lp-PLA2 mass and activity levels decreased. The increase in hsCRP, an acute phase protein, likely represents nonspecific inflammation occurring in stroke patients, and this could be attributable to brain injury and inflammation or to secondary infectious and inflammatory complications of stroke, such as urinary tract infections, pneumonias, and decubiti. The increase in hsCRP is consistent with findings in our prognostic study among patients with stroke, in which stroke severity was associated with elevations in hsCRP. In a previous study, we also found that levels of hsCRP are stable for at least 1 month after elevation at the time of stroke.

The decrease in Lp-PLA2 mass and activity, on the other hand, may reflect the colocalization of Lp-PLA2 with LDL. LDL levels also decrease after MI and, to a lesser extent, after stroke. Additional possible reasons for the decline in Lp-PLA2 include the acute phase response itself, with consequent changes in levels of hepatically-produced acute phase proteins, including albumin and fibrinogen. These proteins could affect protein-binding and activity of Lp-PLA2. The fact that Lp-PLA2 levels move in the opposite direction from hsCRP after stroke further confirms that not all inflammation-related markers behave similarly.

Changes in marker levels at the time of stroke may have implications for clinical management. Measurements made soon after stroke and MI may not be reflective of stable pre-event levels. Use of these biomarkers in secondary prevention, therefore, likely requires use of different thresholds to determine risk than in primary prevention. Further studies, ideally multicenter, are needed to determine these thresholds.

In conclusion, Lp-PLA2 mass levels decrease slightly over time and to a greater extent in those on lipid modifying therapy, but their use in defining major risk categories remains relatively stable. HsCRP and LpPLA2 activity appear stable over time. Acutely after stroke and myocardial infarction, however, hsCRP increases, whereas Lp-PLA2 mass and activity levels decrease modestly. These changes imply that measurements made soon after stroke and MI may not be reflective of stable pre-event levels. Use of these biomarkers in secondary prevention likely requires use of different thresholds to determine risk than in primary prevention.

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Disclosures
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References


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