Procalcitonin and Midregional Proatrial Natriuretic Peptide as Markers of Ischemic Stroke
The Northern Manhattan Study

Mira Katan, MD, MS; Yeseon P. Moon, MS; Myunghee C. Paik, PhD; Beat Mueller, MD; Andreas Huber, MD; Ralph L. Sacco, MD, MS; Mitchell S.V. Elkind, MD, MS

Background and Purpose—Chronic infections and neuroendocrine dysfunction may be risk factors for ischemic stroke (IS). We hypothesized that selected blood biomarkers of infection (procalcitonin [PCT]), hypothalamic–pituitary–adrenal axis function (copeptin), and hemodynamic dysfunction (midregional proatrial natriuretic peptide [MRproANP]) are associated with incident IS risk in the multiethnic, urban Northern Manhattan Study (NOMAS) cohort.

Methods—A nested case–control study was performed among initially stroke-free participants. Cases were defined as first IS (n=172). We randomly selected controls among those who did not develop an event (n=344). We calculated Cox proportional hazards models with inverse probability weighting to estimate the association of blood biomarkers with risk of stroke after adjusting for demographic, behavioral, and medical risk factors.

Results—Those with PCT and MRproANP, but not copeptin, in the top quartile, compared with the lowest quartile, were associated with IS (for PCT adjusted hazard ratio [HR], 1.9; 95% confidence interval [CI], 1.0–3.8 and for MRproANP adjusted HR, 3.5; 95% CI, 1.6–7.5). The associations of PCT and MRproANP differed by stroke etiology; PCT levels in the top quartile were particularly associated with small vessel stroke (adjusted HR, 5.1; 95% CI, 1.4–18.7) and MRproANP levels with cardioembolic stroke (adjusted HR, 16.3; 95% CI, 3.7–70.9).

Conclusions—Higher levels of PCT, a marker of infection, and MRproANP, a marker for hemodynamic stress, were independently associated with IS risk. PCT was specifically associated with small vessel and MRproANP with cardioembolic stroke risk. Further study is needed to validate these biomarkers and determine their significance in stroke risk prediction and prevention. (Stroke. 2016;47:1714-1719. DOI: 10.1161/STROKEAHA.115.011392.)

Key Words: biomarker ■ hemodynamic ■ infection ■ risk factor ■ stress ■ stroke

Because traditional risk factors do not account for all strokes, the identification of novel pathways of stroke risk may lead to additional means to reduce burden of disease. Thus, the measurement of blood biomarkers, which reflect underlying pathological pathways, could serve as indicators of novel risk mechanisms. We therefore selected candidate blood biomarkers involved in 3 different pathophysiological processes.

Serum procalcitonin (PCT) concentrations are correlated with extent and severity of bacterial invasion,1 and serological markers of chronic infection were associated with stroke and carotid plaque in prospective studies, even after adjusting for other potential confounding factors.2 Thus, we hypothesized that procalcitonin, as a surrogate for bacterial infections, would be associated with ischemic stroke (IS) and that the magnitude of association would be highest for noncardioembolic stroke.

Chronic activation of the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system may promote pathophysiological conditions, such as atherosclerosis,3 diabetes mellitus,4 and congestive heart failure.5 Copeptin, a hypothalamic stress hormone, has been associated with poor functional outcome and mortality after stroke,6 and stroke after transient ischemic attack.7 Higher natriuretic peptide concentrations in patients with stroke are associated with increased sympathetic activation, higher mortality,8 and in cross-sectional studies with cardioembolic stroke etiology.9 Thus, we hypothesized that copeptin, as a novel marker of neuroendocrine dysfunction, and midregional proatrial natriuretic peptide (MRproANP), as a marker of hemodynamic dysfunction, would also be associated with stroke risk. Furthermore, we hypothesize that MRproANP is specifically associated with cardioembolic stroke.
Methods

Standard Protocol Approvals, Registrations, and Patient Consents
The Institutional Review Boards at Columbia University Medical Center and University of Miami approved the study. All participants gave informed consent to participate.

Source Study Population
The Northern Manhattan Study (NOMAS) is a population-based prospective cohort study designed to evaluate the effects of medical, socioeconomic, and other risk factors on the incidence of vascular disease in a stroke-free multiethnic community. A total of 3298 participants enrolled between 1993 and 2001. Methods of participant recruitment, evaluation, and follow-up have been reported. Briefly, participants were enrolled if they: (1) had never had a stroke, (2) were >40 years of age; and (3) resided in Northern Manhattan for at least 3 months in a household with a telephone. Participants underwent a thorough baseline examination, including comprehensive medical history, physical examination, and review of medical records. Study definitions for race–ethnicity, hypertension, diabetes mellitus, cardiovascular disease, and other risk factors have been described. Trained bilingual research assistants performed interviews; study physicians conducted physical and neurological examinations.

Follow-Up and End Points
Participants were followed annually via telephone to detect new neurological events. Participants who responded positively were scheduled for in-person assessment; the average annual contact rate was 99%. We prospectively screened all admissions and discharges to detect hospitalizations and outcomes not captured by telephone interview.

The primary end point, IS, was defined as the first symptomatic occurrence of fatal or nonfatal IS according to the World Health Organization criteria. Stroke etiology was based on the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria, and adjudicated by a consensus of 2 study neurologists.

Formation of Analytic Cohort
For reasons of cost and efficiency, a case–control design was used for this biomarker analysis. Of the source population of 3298 individuals followed from baseline (1993–2001) until 2013 for incident stroke, blood samples were available for 2428 subjects. Patients with hemorrhages were excluded from this analysis. We identified participants who developed IS (n=172) during follow-up, controls (n=344) were afterward randomly selected among participants who had not developed stroke. We chose the allocation ratio of 1 case to 2 controls based on (1) cost consideration and (2) increase in power given the expected prevalence of exposure among controls. In total, 516 participants were evaluated.

Biomarker Measurements
At baseline, blood samples were obtained, centrifuged, and frozen at −80°C until the time of analysis. The samples of the 516 subjects were shipped on dry ice to a specialized laboratory in Switzerland (Kantonsspital-Aarau). Serum samples were assayed for levels of PCT using a rapid, sensitive assay with a detection limit of 0.02 ng/mL (BRAHMS-PCT sensitive KRYPTOR; Thermo-Scientific, Henningsdorf Berlin, Germany). Copeptin levels were measured by an immunoluminometric assay; the functional assay sensitivity (20% interassay coefficient of variation) of this manual assay is <1 pmol/L (BRAHMS-CT–proA VP LIA). MRproANP levels were also measured using an immunoassay with a detection limit of 2.1 pmol/L (BRAHMS KRYPTOR). Quality control was maintained using standardized procedures, including running samples in duplicate. All testing was performed in batch analyses blinded to clinical data, including outcome. Stability has been documented for all biomarkers.

Statistical Analyses
Descriptive statistics were calculated and compared by cases versus controls using Wilcoxon Rank-sum test for continuous variables and the χ² test for dichotomous variables. The primary outcome was IS, and the secondary outcomes were stroke pathogenesis. The main predictors, PCT, copeptin, and MRproANP, were log transformed to achieve linearity and afterward analyzed by quartile to facilitate clinical interpretation. We fit Cox proportional hazard models with inverse probability weighting to calculate HRs and 95% CIs, unadjusted and adjusted for demographics (model 1) as well as adjusted for demographic and vascular risk factors (model 2). The weighting utilized for the implementation of the inverse probability weighting method included all variables that were included in the final model. Adjusted covariates were predictors of IS in previous analyses in NOMAS and traditionally accepted risk factors for stroke (ie, age, sex, race–ethnicity, education, physical activity, smoking status, diabetes mellitus, hypertension, cardiac disease, low-density lipoprotein, and high-density lipoprotein), including estimated glomerular filtration rate because these biomarkers undergo renal clearance. All testing was 2-tailed, performed using SAS v9.1.3 (SAS Institute, Cary, NC), and P<0.05 was considered statistically significant.

Results

Baseline Characteristics
The median age at baseline for the 172 IS cases was 72 (interquartile range, 65–78) years, which was higher than in controls (68; interquartile range, 60–77 years). Cases were 53% Hispanic, 29% black, and 17% white, comparable with controls. Cases were, as expected, more likely to have hypertension, diabetes mellitus, and cardiac disease than controls. Biomarker levels of interest were higher in cases than in controls; the most prominent difference was observed in MRproANP levels and no significant difference was seen for copeptin (Table 1). Cases had a shorter mean follow-up time (9.8±3.6 years) than controls (13.6±5.9 years).

Stroke pathogeneses were: 29 (17%) larger artery atherosclerotic, 38 (22%) small vessel, 57 (33%) cardioembolic, 41 (24%) cryptogenic, 3 (2%) other determined, and 3 (1%) undetermined pathogenesis because of lack of documentation. Distribution of MRproANP levels differed by stroke etiology. The levels were greater among those with cardioembolic stroke (Table 2). Distribution of other biomarkers, however, did not differ across stroke pathogeneses.

Association of PCT With IS
In the unadjusted analysis, individuals in the top PCT quartile were at increased risk of IS compared with those in the lowest quartile (HR, 2.4; 95% CI, 1.3–4.3). After adjusting for demographic and vascular risk factors, those with PCT in the top quartile, compared with the lowest, remained at increased risk of IS (adjusted HR, 1.9; 95% CI, 1.0–3.8, Table 3). In an analysis among a subgroup with data available on infectious burden, as well as with, there was no material change in the results.

In analyses for each stroke etiology considered separately, individuals in the top PCT, compared with the lowest quartile, were at increased risk for small vessel strokes (adjusted HR, 5.1; 95% CI, 1.4–18.7, Table 4), but not for cardioembolic (adjusted HR, 2.1; 95% CI, 0.6–6.7) or larger artery atherosclerotic stroke (adjusted HR, 1.1; 95% CI, 0.2–6.2).
Association of Copeptin With IS

In the unadjusted analysis, individuals in the top copeptin quartile were at increased risk of IS compared with those in the lowest quartile (HR, 1.2; 95% CI, 1.0–1.5). After adjusting (models 1 and 2), however, copeptin was no longer associated with IS risk (Table 3). There were no significant associations of copeptin levels with any stroke pathogeneses.

Association of MRproANP With IS

In the unadjusted analysis, individuals in the top MRproANP quartile were at increased risk of IS compared with those in the lowest (HR, 4.5; 95% CI, 2.6–7.8). This association remained after adjusting for demographic and vascular risk factors with an HR of 3.5 (95% CI, 1.6–7.5; Table 3).

Individuals in the top MRproANP quartile were at increased risk of cardioembolic (adjusted HR, 16.3; 95% CI, 3.7–70.9; Table 5) but not small vessel (adjusted HR, 1.4; 95% CI, 0.3–7.4) or larger artery atherosclerotic stroke (adjusted HR, 0.6; 95% CI, 0.1–5.0).

Discussion

In this urban multiethnic population-based sample, we found that PCT, a marker of bacterial infection, and MRproANP, a marker of hemodynamic dysfunction, were independently...
Table 3. Association of Biomarkers With Ischemic Stroke

<table>
<thead>
<tr>
<th>Parameter Cut Off</th>
<th>Unadjusted; HR (95% CI)</th>
<th>Model 1; HR (95% CI)*</th>
<th>Model 2; HR (95% CI)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copeptin</td>
<td></td>
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</tr>
<tr>
<td>First quartile, &lt;3.9 pmol/L</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second quartile, 3.9–6.6 pmol/L</td>
<td>0.8 (0.5–1.5)</td>
<td>0.7 (0.4–1.4)</td>
<td>0.8 (0.4–1.6)</td>
</tr>
<tr>
<td>Third quartile, 6.7–11.5 pmol/L</td>
<td>0.7 (0.7–2.1)</td>
<td>1.0 (0.6–1.9)</td>
<td>1.2 (0.6–2.1)</td>
</tr>
<tr>
<td>Fourth quartile &gt;11.5 pmol/L</td>
<td>1.6 (0.9–2.8)</td>
<td>1.2 (0.7–2.2)</td>
<td>1.1 (0.6–2.2)</td>
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<tr>
<td>Midregional proatrial natriuretic peptide</td>
<td></td>
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<tr>
<td>First quartile, &gt;58.4 pmol/L</td>
<td>Reference</td>
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<tr>
<td>Second quartile, 58.4–91.1 pmol/L</td>
<td>1.5 (0.9–2.8)</td>
<td>1.5 (0.8–2.9)</td>
<td>1.3 (0.7–2.5)</td>
</tr>
<tr>
<td>Third quartile, 91.2–144.8 pmol/L</td>
<td>1.8 (0.9–3.1)</td>
<td>1.6 (0.8–3.0)</td>
<td>1.6 (0.8–3.1)</td>
</tr>
<tr>
<td>Fourth quartile &gt;144.8 pmol/L</td>
<td>4.5 (2.6–7.8)</td>
<td>3.9 (1.9–7.6)</td>
<td>3.5 (1.6–7.5)</td>
</tr>
<tr>
<td>PCT</td>
<td></td>
<td></td>
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<tr>
<td>First quartile, &lt;0.02 μg/L</td>
<td>Reference</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second quartile, 0.02–0.03 μg/L</td>
<td>1.9 (1.1–3.4)</td>
<td>1.7 (0.9–3.2)</td>
<td>1.7 (0.9–3.4)</td>
</tr>
<tr>
<td>Third quartile, 0.031–0.05 μg/L</td>
<td>1.7 (0.9–3.2)</td>
<td>1.6 (0.8–2.9)</td>
<td>1.8 (0.9–3.5)</td>
</tr>
<tr>
<td>Fourth quartile &gt;0.05 μg/L</td>
<td>2.4 (1.3–4.3)</td>
<td>2.1 (1.1–3.9)</td>
<td>1.9 (1.0–3.8)</td>
</tr>
</tbody>
</table>

Cl indicates confidence interval; HR, hazard ratio; and PCT, procalcitonin.
*Model 1: adjusted for demographics (ie, age, sex, race–ethnicity, and education).
†Model 2: adjusted for age, sex, race–ethnicity, education, physical activity, smoking status, diabetes mellitus, hypertension, cardiac disease, low-density lipoprotein, high-density lipoprotein, and estimated glomerular filtration rate.

Table 4. Association of PCT With Risk of Small Vessel Stroke*

<table>
<thead>
<tr>
<th>PCT</th>
<th>Hazard Ratio (95% Confidence Interval)*</th>
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</thead>
<tbody>
<tr>
<td>First quartile</td>
<td>Reference</td>
</tr>
<tr>
<td>Second quartile</td>
<td>2.4 (0.6–10.1)</td>
</tr>
<tr>
<td>Third quartile</td>
<td>1.7 (0.2–11.3)</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>5.1 (1.4–18.7)</td>
</tr>
</tbody>
</table>

PCT indicates procalcitonin.
*The case group for this analysis includes only those with small vessel stroke, and those with other strokes were excluded.

Table 5. Association of MRproANP With Risk of Cardiogenic Stroke*

<table>
<thead>
<tr>
<th>MRproANP</th>
<th>Hazard Ratio (95% Confidence Interval)†</th>
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<tbody>
<tr>
<td>First quartile</td>
<td>Reference</td>
</tr>
<tr>
<td>Second quartile</td>
<td>1.3 (0.3–5.2)</td>
</tr>
<tr>
<td>Third quartile</td>
<td>4.2 (1.1–15.5)</td>
</tr>
<tr>
<td>Fourth quartile</td>
<td>16.3 (3.7–70.9)</td>
</tr>
</tbody>
</table>

MRproANP indicates midregional proatrial natriuretic peptide.
*The case group for this analysis includes only those with cardiogenic stroke, and those with other strokes were excluded.
†Adjusted for age, sex, race–ethnicity, education, physical activity, smoking status, diabetes mellitus, hypertension, cardiac disease, low-density lipoprotein, high-density lipoprotein, and estimated glomerular filtration rate.

associated with IS risk. PCT was specifically associated with small vessel stroke and MRproANP with cardioembolic stroke. Copeptin, a hypothalamic stress hormone, which has been shown to be a promising candidate for risk stratification in the acute phase after stroke,6,7 was not associated with incident stroke in this cohort.

Basic and clinical research provide evidence that inflammation triggered by infectious agents may play a role in the pathogenesis of IS.2 In previous analyses of this population, a weighted measure of infectious burden including several pathogens was associated with stroke risk, carotid artery atherosclerosis, and cognitive impairment.3,17,18 PCT synthesis and secretion are upregulated by bacterial toxins and certain bacteria-specific proinflammatory mediators (eg, interleukin-1β, tumor necrosis factor-α), and interleukin-6).19,20 Administration of exogenous PCT to septic animals significantly increased the mortality rate compared with control animals; thus PCT seems to display immunologic properties also as a bioactive molecule.21 The importance of PCT under homeostatic conditions in the general population has not been studied extensively. In the Malmö Diet and Cancer cohort, PCT was independently associated with risk for all-cause and cancer mortality in apparently healthy men.22 In a subpopulation of the same cohort, PCT was also associated with the incidence of coronary events and vascular death including stroke, but this association did not remain significant after adjusting for known vascular risk factors,23 probably because of lack of power.

In this multiethnic urban cohort of individuals with no previous stroke history, we found a link between higher circulating PCT concentrations and an increased risk specifically of small vessel stroke. Chronic infections have also been implicated in development of cognitive impairment and dementia that may have underlying small vessel disease mechanisms.17 The biological role of PCT in vivo at low concentrations in apparently healthy individuals has, to date, been unexplored. On the basis of our data, we hypothesize that plasma PCT reflects ongoing subclinical inflammatory processes triggered by bacterial endotoxins.

We do not have an obvious explanation for why PCT was specifically associated with small vessel disease and not with large vessel disease. It might be that the inflammatory processes involved in large and small vessel disease differ, and that PCT reflects one of these processes more strongly, but we cannot clarify this based on our observational data.

A-type natriuretic peptide (ANP) is a member of the family of natriuretic peptides. Its physiological role is mainly the regulation of blood pressure ascribed to its natriuretic, diuretic, and vasodilating action. ANP has emerged as reliable
prognostic marker for congestive heart failure, risk of cardiovascular death, and stroke outcome.\textsuperscript{9,24} ANP has also been shown to help in identifying cardioembolic stroke pathogenesis in cross-sectional studies.\textsuperscript{9,25,26} Related proteins, such as N-terminal pro-B-natriuretic peptide, have been associated with incident stroke in some studies.\textsuperscript{27,28} The pathophysiological mechanism explaining the independent association of MRproANP with IS specifically of cardioembolic origin may reflect the fact that high MRproANP concentrations indicate the presence not only of manifest heart failure but also of early cardiac pathology, including atrial cardiopathy,\textsuperscript{29} leading to embolism.

Copeptin, a hypothalamic stress marker, has been shown to improve risk stratification after acute IS and transient ischemic attack in several studies.\textsuperscript{6,7,30} Copeptin measured in the German Diabetes Dialysis Study was associated with increased risk for stroke sudden death, other cardiovascular events, and mortality.\textsuperscript{31} In diabetic individuals from the Malmö Diet and Cancer study, copeptin was associated with the combined end point of coronary artery disease, heart failure, and death.\textsuperscript{32} In a subcohort of the Malmö Diet and Cancer study, quartiles of copeptin had dose–response relationships with the odds of developing diabetes mellitus, even after additionally adjusting for baseline fasting glucose and insulin.\textsuperscript{4} There is, however, less data available on the role of copeptin as prognostic marker for stroke in apparently healthy subjects. Our study also did not find associations of copeptin with IS risk. This lack of association could reflect the fact that chronic stress does not influence copeptin expression in the same way as acute stress and that other vascular risk factors are more important for development of IS.

Our study has limitations. First, the blood samples were stored at $-80^\circ\text{C}$ for several years, which could lead to protein degradation. However, all assessed analytics are stable when stored at $-70^\circ\text{C}$ and degradation would have affected cases and controls alike. Second, considering costs and efficiency we chose a case–control study design, which is more prone to bias than a prospective cohort study design; however, we used the inverse probability weighting method to correct for potential selection bias. Moreover, because of the study design and the pilot character of the data (relatively small numbers) we cannot reliably assess the additive predictive value of these markers using measures of reclassification. However, this was also not the primary aim of this study. As a first step, we wanted to gain insight into potential mechanisms of novel stroke risk biomarkers. Future studies in larger prospective cohorts are needed to ascertain clinical use of these biomarkers and their incremental value over existing clinical risk prediction schemes. Finally, as the incidence rate of larger artery atherosclerotic and CE strokes is relatively small, the results for subtypes should be considered cautiously and need further external validation.

The strengths of this study include the population-based multiethnic cohort, including a large proportion of Hispanics who are frequently underrepresented in other cohort studies, minimal loss to follow-up, and the ability to adjust for numerous potential covariates. Moreover, we were able to assess stroke pathogenesis, and to correct selection biases using inverse probability weighted method. Finally, we report a potential novel role of PCT and MRproANP in the development of incidence stroke.

If our results are confirmed, this could have clinical implications. On a population level, for example, people at higher stroke risk based on their risk factor profile who also had higher MRproANP levels could be monitored more closely about cardiac disease, whereas those with higher PCT levels could undergo preventive vaccinations for common infections. Clinical trials using these biomarkers would be needed, however, to test such approaches.

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**References**
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