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Procalcitonin and Midregional-proAtrial Natriuretic Peptide as markers of ischemic stroke: the Northern Manhattan Study

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Abstract

Background and Purpose—Chronic infections and neuroendocrine dysfunction may be risk factors for ischemic stroke. We hypothesized that selected blood biomarkers of infection (procalcitonin, or PCT), hypothalamic-pituitary-axis function (copeptin), and hemodynamic dysfunction (midregional-pro-atrial natriuretic peptide, or MRproANP) are associated with incident ischemic stroke 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 ischemic stroke (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 to the lowest quartile, were associated with ischemic stroke (for PCT adjusted HR 1.9, 95% CI 1.0–3.8; 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).

Conclusion—Higher levels of procalcitonin, a marker of infection, and MRproANP, a marker for hemodynamic stress, were independently associated with ischemic stroke risk. PCT was specifically associated with small vessel and MRproANP with cardioembolic stroke risk. Further

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study is needed to validate these biomarkers and determine their significance in stroke risk prediction and prevention.

Search Terms

stroke; copeptin; pct; MRproANP; risk factor

Introduction

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 three different pathophysiological processes.

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

Chronic activation of the hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system may promote pathophysiological conditions like atherosclerosis, diabetes mellitus, and congestive heart failure. Copeptin, a hypothalamic stress hormone, has been associated with poor functional outcome and mortality after stroke, and stroke after transient ischemic attack. Higher natriuretic peptide concentrations in stroke patients are associated with increased sympathetic activation, higher mortality and in cross-sectional studies with cardioembolic stroke etiology. Thus we hypothesized that copeptin, as a novel marker of neuroendocrine dysfunction, and MRproANP, as a marker of hemodynamic dysfunction, would also be associated with stroke risk. Further 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 1993–2001. 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 over age 40 years; and (3) resided in Northern Manhattan for at least 3

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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, cardiac disease, and other risk factors have been described. Trained bilingual research assistants performed interviews; study physicians conducted physical and neurological examinations.

Follow up and endpoints

Participants were followed annually via telephone to detect new neurologic 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 endpoint, ischemic stroke, was defined as the first symptomatic occurrence of fatal or non-fatal ischemic stroke according to the World Health Organization criteria. Stroke etiology was based on the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria, and adjudicated by a consensus of two 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, and controls (n=344) were afterwards 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, Germany). Copeptin levels were measured by an immunoluminometric assay; the functional assay sensitivity (20% inter-assay coefficient of variation) of this manual assay is <1 pmol/L (BRAHMS-CT-proAVP 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 vs. controls using Wilcoxon rank sum test for continuous variables and the *chi*-squared test for dichotomous variables. The primary outcome was IS and the secondary outcomes were stroke etiologies. The main predictors, PCT, copeptin and MRproANP, were log transformed to achieve linearity and

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afterwards analyzed by quartile in order to facilitate clinical interpretation. We fit Cox proportional hazard models with inverse probability weighting to calculate hazard ratios and 95% confidence intervals (HR, 95% CI), 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 prior analyses in NOMAS and traditionally accepted risk factors for stroke (i.e. age, sex, race-ethnicity, education, physical activity, smoking status, diabetes mellitus, hypertension, cardiac disease, low density lipoprotein (LDL), high density lipoprotein (HDL)), including estimated glomerular filtration rate since these biomarkers undergo renal clearance. All testing was two-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 (IQR) 65–78) years, which was higher than in controls (68, IQR 60–77 years). Cases were 53% Hispanic, 29% black, and 17% white, comparable to controls. Cases were, as expected, more likely to have hypertension, diabetes, and cardiac disease than controls. Biomarker levels of interest were higher in cases than 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 etiologies were: 29 (17%) LAA, 38 (22%) small vessel, 57 (33%) cardioembolic, 41 (24%) cryptogenic, 3 (2%) other determined, and 3 (1%) undetermined etiology due to 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 etiologies.

Association of PCT with IS

In the unadjusted analysis, individuals in the top PCT quartile were at increased risk of IS compared to 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 to the lowest, remained at increased risk of IS (adjusted HR 1.9, 95% CI 1.0–3.8, see table 3). In an analysis among a subgroup with data available on infectious burden, as well, there was no material change in the results.

In analyses for each stroke etiology considered separately, individuals in the top PCT, compared to the lowest quartile, were at increased risk for small vessel strokes (adjusted HR 5.1, 95% CI 1.4–18.7, see table 4), but not for cardioembolic (adjusted HR 2.1, 95% CI 0.6–6.7) or LAA stroke (adjusted HR 1.1, 95% CI 0.2–6.2).

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Association of copeptin with IS

In the unadjusted analysis, individuals in the top copeptin quartile were at increased risk of IS compared to those in the lowest quartile (HR 1.2, 95% CI 1.0–1.5). After adjusting (model 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 etiologies.

Association of MRproANP with IS

In the unadjusted analysis, individuals in the top MRproANP quartile were at increased risk of IS compared to 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, see table 3).

Individuals in the top MRproANP quartile were at increased risk of cardioembolic (adjusted HR 16.3, 95% CI 3.7–70.9, see table 5) but not small vessel (adjusted HR 1.4, 95% CI 0.3–7.4) or LAA 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 associated with ischemic stroke 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[9], 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. In prior analyses of this population, a weighted measure of infectious burden (IB) including several pathogens was associated with stroke risk, carotid artery atherosclerosis, and cognitive impairment[10,11]. PCT synthesis and secretion are up-regulated by bacterial toxins and certain bacteria-specific pro-inflammatory mediators (e.g., interleukin-1b, tumor necrosis factor-[alpha], and interleukin-6)[9]. Administration of exogenous PCT to septic animals significantly increased the mortality rate compared to control animals; thus PCT seems to display immunological properties also as a bioactive molecule. 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. 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, probably due to 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. The biological role of PCT *in vivo* at low concentrations in apparently healthy individuals has so far been unexplored. Based on our data we hypothesize that plasma PCT

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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[,] . A-type natriuretic peptide has also been shown to help in identifying cardioembolic stroke etiology in cross-sectional studies[,,] . Related proteins, such as N-terminal pro-B-natriuretic peptide (NTproBNP), have been associated with incident stroke in some studies[,] . 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 early cardiac pathology, including atrial cardiopathy, leading to embolism.

Copeptin, a hypothalamic stress marker, has been shown to improve risk stratification after acute ischemic stroke and TIA in several studies[,,] . Copeptin measured in the German Diabetes Dialysis Study, was associated with increased risk for stroke sudden death, other cardiovascular events, and mortality. In diabetic individuals from the Malmö Diet and Cancer study, copeptin was associated with the combined endpoint of coronary artery disease, heart failure and death. In a sub-cohort of the Malmö Diet and Cancer study, quartiles of copeptin had dose-response relationships with the odds of developing diabetes, even after additionally adjusting for baseline fasting glucose and insulin. 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 °C for several years, which could lead to protein degradation. However, all assessed analytes are stable when stored at −70°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, due to 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 utility of these biomarkers and their incremental value over existing clinical risk prediction schemes. Finally, as the incidence rate of LAA and CE strokes is relatively small, the results for subtypes should be considered cautiously and need further external validation.

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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 etiology, 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 regarding cardiac disease, while 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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Table 1

Baseline characteristics:

Parameters	Stroke cases N=172	Randomly selected stroke-free controls N=344	p-value[§]
Sociodemographic factors			
Age (years), median (IQR)	72 (65–78)	68 (60–77)	<0.01
Female sex, n (%)	102 (59%)	224 (65%)	ns
Race-Ethnicity Non-Hispanic White, n (%) Non-Hispanic Black, n (%) Hispanic, n (%) Other, n (%)	30 (17%) 49 (29%) 91 (53%) 2 (1%)	63 (18%) 78 (23%) 195 (57%) 8 (2%)	ns
Medicaid or no insurance, n (%)	79 (46%)	164 (47%)	ns
High school education, n (%)	69 (40%)	157 (46%)	ns
Risk factors			
Systolic BP(mm Hg), median (IQR)	150 (137–161)	140 (130–155)	<0.01
Hypertension[*], n (%)	147 (85%)	225 (74%)	<0.01
Cardiac disease[**], n (%)	59 (34%)	78 (23%)	<0.01
Diabetes mellitus, n (%)	69 (40%)	85 (25%)	<0.01
Any physical activity, n (%)	96 (56%)	186 (54%)	ns
Past smoker, n (%)	73 (43%)	123 (36 %)	ns
Current smoker, n (%)	32 (18%)	57 (16%)	ns
Laboratory measurements			
Low density lipoprotein mg/dL, median (IQR)	124 (107–149)	128 (106–149)	ns
High density lipoprotein mg/dL, median (IQR)	44 (35–54)	44 (36–57)	ns
Estimated glomerular filtration rate, median (IQR)	76 (63–90)	78(64–92)	ns
Procalcitonin, ug/L, median (IQR)	0.032 (0.024–0.049)	0.029 (0.019–0.043)	<0.05
Copeptin, pmol/L, median (IQR)	7.2 (4.1–11.9)	6.4 (3.9–10.5)	ns
MRproANP, pmol/L, median (IQR)	110 (69–181)	85 (55–134)	<0.01

IQR: interquartile range

[*] Hypertension defined as: history, taking medications, or systolic blood pressure ≥140mmHg, or diastolic blood pressure ≥90mmHg;

[**] Any cardiac disease.

[§] Wilcox rank sum test for continuous measures and Chi-Squared test for categorical measures, respectively

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Table 2

Distribution of biomarker levels by stroke etiology

Biomarker	Stroke Etiology	Q1	Median	Q3
Copeptin pmol/L	Large-artery atherosclerosis[1]	4.1	6.5	11.5
	Small-artery occlusion[2]	4.1.	7.2	11.9
	Cardioembolism[3]	5.4	7.9	13.5
	Cryptogenic, other and undermined[4]	3.5	7.1	11.1
MRproANP pmol/L	Large-artery atherosclerosis[1]	52	85	137
	Small-artery occlusion[2]	51	93	156
	Cardioembolism[3]	97	157	195
	Cryptogenic, other and undermined[4]	69	97	174
PCT ug/L	Large-artery atherosclerosis[1]	0.024	0.039	0.048
	Small-artery occlusion[2]	0.023	0.031	0.059
	Cardioembolism[3]	0.024	0.030	0.047
	Cryptogenic, other and undermined[4]	0.023	0.032	0.045

Stroke subgroups according to etiologies were:

[1] Large-artery atherosclerosis n=29 (17%);

[2] Small-artery occlusionn=38 (22%);

[3] Cardioembolic n=57 (33%);

[4] cryptogenic n=41, other determined n=3 and undetermined etiology due to lack of documentation n=3, amounting to n= 47(28%)

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Table 3

Association of biomarkers with ischemic stroke

Parameter Cut off	Unadjusted HR (95% CI)	Model 1* HR (95% CI)	Model 2** HR (95% CI)
Copeptin			
First quartile <3.9 pmol/L	Reference		
Second quartile 3.9–6.6 pmol/L	0.8 (0.5–1.5)	0.7 (0.4–1.4)	0.8 (0.4 – 1.6)
Third quartile 6.7– 11.5 pmol/L	0.7 (0.7–2.1)	1.0 (0.6–1.9)	1.2 (0.6 – 2.1)
Fourth quartile >11.5 pmol/L	1.6 (0.9–2.8)	1.2 (0.7–2.2)	1.1 (0.6 – 2.2)
Mid Regional pro-Atrial Natriuretic Peptide			
First quartile > 58.4 pmol/L	Reference		
Second quartile 58.4–91.1 pmol/L	1.5 (0.9–2.8)	1.5 (0.8–2.9)	1.3 (0.7 – 2.5)
Third quartile 91.2–144.8 pmol/L	1.8 (0.9–3.1)	1.6 (0.8–3.0)	1.6 (0.8 – 3.1)
Fourth quartile >144.8 pmol/L	4.5 (2.6–7.8)	3.9 (1.9–7.6)	3.5 (1.6 – 7.5)
Procalcitonin			
First quartile < 0.02 ug/L	Reference		
Second quartile 0.02– 0.03 ug/L	1.9 (1.1–3.4)	1.7 (0.9–3.2)	1.7 (0.9 – 3.4)
Third quartile 0.031–0.05 ug/L	1.7 (0.9–3.2)	1.6 (0.8–2.9)	1.8 (0.9 – 3.5)
Fourth quartile >0.05 ug/L	2.4 (1.3–4.3)	2.1 (1.1–3.9)	1.9 (1.0 – 3.8)

*Model 1; adjusted for demographics (i.e. age, sex, race-ethnicity, 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, estimated glomerular-filtration-rate

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Table 4

Association of PCT with risk of small vessel stroke[$]

PCT	Hazard Ratio* (95% Confidence Interval)
First quartile	Reference
Second quartile	2.4 (0.6–10.1)
Third quartile	1.7 (0.2–11.3)
Fourth quartile	5.1 (1.4–18.7)

[$]The case group for this analysis includes only those with small vessel stroke, and those with other strokes were excluded.

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Table 5

Association of MRproANP with risk of cardioembolic stroke[¥]

MRproANP	Hazard Ratio[*] (95% Confidence Interval)
First quartile	Reference
Second quartile	1.3 (0.3 – 5.2)
Third quartile	4.2 (1.1– 15.5)
Fourth quartile	16.3 (3.7 – 70.9)

[¥] The case group for this analysis includes only those with cardioembolic 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 (LDL), high density lipoprotein (HDL), estimated glomerular-filtration-rate (eGFR).