N-terminal pro-B-type natriuretic peptide and risk of future cognitive impairment in the REGARDS cohort

Running Title: NT-proBNP and risk of cognitive impairment

Mary Cushman, MD, MSc*; Peter W Callas, PhD*; Leslie A. McClure, PhD†; Frederick W. Unverzagt, PhD‡; Virginia J. Howard, PhD§; Sarah R. Gillett, MD, PhD*; Evan L. Thacker, PhD|; Virginia G. Wadley, PhD§

* Departments of Medicine (MC, SRG), Pathology (MC) and Biometry (PWC), and Cardiovascular Research Institute of Vermont, University of Vermont, Burlington, VT
† Department of Epidemiology & Biostatistics, Drexel University, Philadelphia, PA.
‡ Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN
§ Departments of Epidemiology (VJH) and Medicine (VGW), University of Alabama at Birmingham, Birmingham, AL
| Department of Health Science, Brigham Young University, Provo, UT

Address for Correspondence/Reprints: Mary Cushman, MD, MSc, Departments of Medicine and Pathology, University of Vermont, 360 South Park Dr, Colchester, VT 05446. mary.cushman@uvm.edu Phone: 802-656-8968, Fax: 802-656-8965

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Background: Improved understanding of the etiology of cognitive impairment is needed to develop effective preventive interventions. Higher amino-terminal pro-B-type natriuretic peptide (NT-proBNP) is a biomarker of cardiac dysfunction associated with risk cardiovascular diseases and stroke in apparently healthy people.

Objective: To study the association of NT-proBNP with risk of incident cognitive impairment.

Methods: The Reasons for Geographic and Racial Differences in Stroke is a national cohort study of 30,239 black and white Americans age 45 and older at baseline, enrolled in 2003-7. Among participants without prebaseline stroke or cognitive impairment, baseline NT-proBNP was measured in 470 cases of incident cognitive impairment and 557 controls. Cases were participants scoring below the 6th percentile of demographically-adjusted means on at least 2 of 3 serially administered tests (word list learning, word list recall and semantic fluency) over 3.5 years follow-up.

Results: Adjusting for age, gender, race, region of residence, education and income there was an increased odds ratio of incident cognitive impairment with increasing NT-proBNP; participants in the 4th versus 1st quartile (>127 versus ≤33 pg/ml) had a 1.69-fold increased odds (95% CI 1.11-2.58). Adjustment for cardiovascular risk factors and presence of an apolipoprotein E4 allele had no substantial impact on the odds ratio. Results did not differ by age, race, gender or presence of an apolipoprotein E4 allele.

Conclusion: Higher NT-pro-BNP was associated with incident cognitive impairment in this prospective study, independent of atherogenic and Alzheimer's disease risk factors. Future work should clarify pathophysiologic connections of NT-proBNP and cognitive dysfunction.

Key Words: Cognition disorders; biomarkers; risk factors; prospective study; Natriuretic peptide, brain

Introduction

Over 5 million people in the United States are cognitively impaired, and each year 12% of these will develop dementia[1]. As there are no effective treatments, identification of risk factors for cognitive impairment is crucial to developing prevention strategies[2]. Emerging evidence suggests that cardiovascular risk factors are related to increased risk of dementia[2, 3].

N-terminal pro-B-type Natriuretic peptide (NT-proBNP) is a marker of cardiac function used in detection and monitoring of congestive heart failure. Higher NT-proBNP is also a risk marker for future stroke, coronary heart disease and atrial fibrillation[4-10]. Levels are higher with increasing age and in the presence of heart disease, atrial fibrillation and kidney disease[10]. Given the similar risk factors for vascular disease and cognitive impairment, and associations of heart failure and Framingham Stroke Risk Scale scores with cognitive impairment[11, 12], we hypothesized that higher NT-proBNP would be associated with risk of cognitive impairment.

We addressed this hypothesis by studying the association of baseline NT-proBNP with risk of incident cognitive impairment using a nested case-control study design in the Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort study of 30,239 black and white Americans age 45 and older.

Materials and Methods

Participants

The REGARDS cohort is a population-based cohort study investigating racial and geographic disparities in stroke and cognitive impairment[13]. The study enrolled 30,239 individuals age 45 and older by telephone from 2003 to 2007. Blacks and residents of the stroke belt states of North Carolina, South Carolina, Georgia, Tennessee, Mississippi, Alabama, Louisiana, and Arkansas were oversampled, with final proportions of 45% men, 55% women, 58% whites, 42%

blacks, 56% stroke belt residents and 44% non-stroke belt residents. Race was self-identified by participants. Demographic, socioeconomic factors, medical history and verbal informed consent were obtained by computer-assisted telephone interview. At a subsequent in-home examination written informed consent, physical exam, blood samples, electrocardiogram (ECG), and medication inventory were obtained[13]. Study methods were reviewed and approved by Institutional Review Boards at each study institution.

Measurements and Definitions

Baseline cognitive status was determined with the Six-Item Screener (SIS; 3 temporal orientation items and delayed recall of 3 objects) which was validated in community and clinical samples including large numbers of black participants[14]. Scores are 0-6 with 4 or fewer correct indicating cognitive impairment. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic pressure ≥90 mmHg, or self-reported hypertension with use of anti-hypertensive medications. Diabetes was defined by self-report with use of anti-diabetic medications, fasting glucose >126 mg/dL, or non-fasting glucose >200 mg/dL. Albuminuria was defined as urinary albumin to creatinine ratio >30 mg/g. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease (CKD) Epidemiology Collaboration formula, with CKD defined as eGFR <60 ml/min/1.73m^2.[15] Left ventricular hypertrophy (LVH) was classified by centrally-read ECG. Atrial fibrillation was defined as self-report or presence on ECG. Prebaseline heart disease was defined as self-reported myocardial infarction, bypass, angioplasty or stenting, or myocardial infarction on ECG. Prebaseline stroke was defined by self-report of a physician diagnosis. Stroke after baseline was identified and validated as previously described[16].

Longitudinal Cognitive Assessment

Participants were contacted every 6 months to ascertain health status and conduct cognitive testing. Starting in 2006 a three test battery was performed every 2 years including Word List Learning, Word List Recall and Semantic Fluency (animals), all from the Consortium to Establish a Registry for Alzheimer's Disease[17]. The Word List Learning score is the number of words recalled on a 10-item, three-trial word list learning task (range 0-30). Word List Recall is the number of words recalled after a filled delay (range 0-10). Semantic Fluency is the number of animals named in 60 seconds. All testing was supervised with quality control monitoring.

Case Control Study Design

A nested case control study was employed to select a subset of REGARDS participants for NT-proBNP measurement and provide results approximating those obtained from NT-proBNP measurement in the entire cohort. Detailed methods were previously reported [18]. Briefly, after excluding participants with prevalent stroke (n=1930), cognitive impairment at baseline SIS (n=2,319), missing data (n=546), stroke prior to first SIS (n=28) or with insufficient follow-up cognitive testing at the time of sample selection (n=7786), we identified cases from the remaining 17,630 participants. Incident cognitive impairment was defined using an absolute score approach[19] that incorporated regression-based adjustment for age, education, race and gender on the three test cognitive battery. Incident cognitive impairment was defined as scores on 2 of 3 components of the most recently administered three test battery that were $<6^{th}$ percentile of each participant's age, race and gender-predicted score. This identified 495 cases of incident cognitive impairment.

Controls were selected from a cohort random sample of 1100 participants selected from the entire REGARDS cohort for a case-cohort study of stroke[10]. This sample was chosen at random within age, gender and race strata (50% black, 50% white, 50% women, 50% men, and age groups 20% 45-54, 20% 55-64, 25% 65-74, 25% 75-84, and 10% ≥85). After exclusions

using the same criteria for cases, those with insufficient cognitive testing to determine case status, or who became a case, the control group included 587 participants[18]. We did not apply incidence density sampling to potential controls.

Fasting baseline blood samples were drawn, processed, shipped to the University of Vermont, and stored[20]. NT-proBNP was measured in the case control sample using an electrochemiluminescence immunoassay (Roche Elecsys 2010; CV <5%). NT-proBNP was missing on 25 cases and 30 controls due to missing blood samples or technical issues leaving 470 cases and 557 controls for analysis. Apolipoprotein E (ApoE) genotype was determined by TaqMan analysis of rs429358 and rs4712.

Statistical Methods

All analyses were weighted to account for the stratified selection of the controls. We calculated distributions of baseline characteristics (mean/SD or proportion) by case control status. To estimate relative risks, odds ratios and 95% confidence intervals for the association of NT-proBNP concentration and cognitive impairment were calculated using weighted logistic regression. NT-proBNP was divided into quartiles based on the distribution in the control group, with the bottom quartile as the reference group. A p value for trend in odds ratios across quartiles was calculated using a linear contrast statement for quartiles within the SAS surveylogistic procedure. Four levels of adjustment were used to evaluate confounding based on known correlates of NT-proBNP and cognitive function. Model 1 included age, gender, race, region of residence, education and income. Model 2 added hypertension medication use, lipid levels, diabetes, systolic blood pressure, LVH, history of heart disease, atrial fibrillation, congestive heart failure status, alcohol use and physical activity. Model 3 added eGFR and albuminuria. Model 4 added presence of at least one ApoE4 allele. Sensitivity analysis further adjusted for development of incident stroke during follow-up. Model 1 was used for interaction

testing to determine if associations of NT-proBNP quartiles with risk of cognitive impairment differed by age (as a continuous variable and in strata of 45-55, 55-70 and >70 years), race, gender, region of residence and presence of an ApoE4 allele. In the case of a cross product interaction term p-value of NT-proBNP quartile with any of these factors of <0.10, stratified analyses were performed.

Results

The median time from baseline to the most recent three test battery was 3.5 years among the 470 cases and 557 controls. Table 1 shows associations of baseline risk factors with case-control status. Because of the demographically-adjusted regression-based selection of cognitive impairment cases, cases and controls were similar by age, race, gender, and education. Cases were more likely than controls to reside in the stroke belt, have lower income, higher body-mass index, and to have diabetes, hypertension, kidney disease and cardiovascular conditions. They were more likely to smoke and less likely to have moderate or heavy alcohol use. In controls the median NT-proBNP declined with increasing number of APOe4 alleles (71, 52, and 37 pg/ml for 0, 1 or 2 E4 alleles; p trend = 0.007). In cases these values were 84, 88, and 62 pg/ml; p trend = 0.68.

Table 2 shows associations of NT-proBNP with risk of cognitive impairment. In Model 1, NT-proBNP in the top compared to bottom quartile was associated with a 1.69-fold increased risk of cognitive impairment (95% CI 1.11-2.58). Associations did not change after additional adjustment for cardiovascular and lifestyle risk factors for cognitive impairment or with adjustment for presence of at least one ApoE4 allele (Models 2-4). The p-value for trend across quartiles was ≤0.01 in all models although there was a lower risk in the second quartile compared to the first that was not statistically significant. Sensitivity analysis adding adjudicated stroke during follow-up (n=34) to the final model had a small impact on the odds ratios (4^{th} vs 1^{st}

quartile odds ratio 1.56 (95% CI 0.92-2.64), p for trend across quartiles 0.02). Sensitivity analysis excluding participants with NT-proBNP above the manufacturer's cutpoint for heart failure reduced the odds ratio for the top quartile modestly (for model 2: 1.59 (95% CI 0.93-2.71); for model 3: 1.46 (95% CI 0.83-2.54); respective p for trend across quartiles 0.02 and 0.05).

Interaction testing revealed that the association of NT-proBNP with incident cognitive impairment only differed by region (p interaction 0.09). As shown in figure 1, this difference by region was driven by a regional difference in the magnitude of the association for the 3rd quartile. Interaction term p values for NT-proBNP with age, age strata, race, gender and presence of an ApoE4 allele were 0.78, 0.45, 0.16, 0.18 and 0.16, respectively.

Discussion

In this prospective observational study including black and white men and women aged 45 and older, baseline NT-proBNP concentration in the top compared to the bottom quartile was associated with a 1.6-fold increased risk of incident cognitive impairment. Findings were not explained by adjustment for a variety of factors including socio-demographic factors, cardiovascular and lifestyle factors, kidney function measures, occurrence of stroke during follow-up, or presence of an apoE4 allele (which was associated with lower NT-proBNP in controls but not cases). Findings suggest that the observed association reflects a pathophysiology relating NT-proBNP to cognitive impairment that is at least partly distinct from both atherosclerosis and Alzheimer's disease, or that facilitates the clinical expression of pre-existing brain pathology. Results were robust across several subgroups. Apparent differences in results by region were likely a chance finding based on the pattern of association observed.

Most previous research on associations of NT-proBNP with cognitive function reported cross-sectional studies, while our study was prospective so can minimize issues of reverse causality of associations. A study of 56 patients with dementia and cardiovascular disease showed cross sectional correlations of BNP with the conceptualization subtest of the Dementia Rating Scale score[21]. In 1066 elderly type 2 diabetics, those with higher NT-proBNP had lower scores on a cognitive battery, but this association was confounded by vascular and diabetes-related risk factors[22]. In the Rancho Bernardo study, higher NT-pro-BNP was correlated with lower performance on the Mini-Mental State Examination (MMSE) and complex sequential tracking, but not a test of semantic fluency[23]. These associations were independent of other risk factors. Two biomarker discovery studies reported that NT-proBNP was associated with cognitive disorders[24, 25]. In one study, among 147 biomarkers, apoE genotype and NT-proBNP were primary contributors to improved specificity for a diagnosis of Alzheimer's Disease, but NT-proBNP was not associated with apoE genotype [25], contrary to findings here. In 4,029 older community-dwelling adults in Iceland, NT-proBNP was inversely associated with memory, processing speed and executive functioning, as well as lower brain volumes by magnetic resonance imaging; the associations with executive function and processing speed were independent of cardiac output[26].

We are aware of four prospective studies on this topic. In the Alzheimer's Disease Neuroimaging Initiative, biomarker panels including NT-proBNP did not distinguish progression from mild cognitive impairment to Alzheimer's disease over 2 years[27]. In 464 Finnish subjects aged 75 and older, higher baseline NT-proBNP was associated with decline in the MMSE score and a 1.53-fold increased risk of dementia (with only 59 cases) over 5 years[28]. In the Rotterdam Study higher baseline NT-proBNP was associated with risk of future dementia and vascular dementia but not Alzheimer's disease; the effect size was similar to the current report (HR 1.52, 95% CI 1.10-2.09 for NT-proBcNP in 4^{th} vs 1^{st} quartile)[29]. In that study higher NT-

proBNP was associated longitudinally with declining information processing speed, but not with change in tests of attention, concentration, verbal fluency or executive function (memory testing was not done). In a Finnish cohort with 13.8 years of follow-up and dementia determined by administrative data, each SD higher baseline NT-prBNP was associated with a 1.3-fold higher risk of dementia, but not among women[30]. The current study adds to these studies by including a large national sample of US blacks and whites from a broad age range, and by including a large number of cases of incident cognitive impairment. Our findings differ in that we did not evaluate dementia as the outcome or changes in individual test scores.

Mechanisms to explain an association of NT-proBNP with cognitive impairment are uncertain. Strong evidence links higher NT-proBNP with atherosclerotic diseases including stroke[5, 10] so atherosclerosis may be the connection. However, the lack of confounding by cardiovascular risk factors observed here, other findings relating NT-proBNP to cerebrospinal fluid Aβ42 level and t-tau/Aβ42 ratios[24], and to improved diagnostic accuracy of apoE genotyping for cognitive disorders[25] suggests a direct effect of NT-proBNP on brain function. A mutation of atrial natriuretic peptide that is associated with vascular risk was also associated with higher NT-proBNP levels, and leads to endothelial cell hyperpermeability, which might lead to atherosclerotic disease[31] or have other direct effects on brain function. Alternatively, NT-proBNP may simply be a marker of cardiac dysfunction that directly or indirectly contributes to cognitive impairment[32]. The observed modest reduction of the odds ratio of cognitive impairment with higher NT-proBNP after excluding participants with NT-proBNP above the manufacturer's cutpoint for heart failure suggests that a partial explanation for our findings could be the impact of unrecognized heart failure impacting cognitive impairment. However, it is also possible that levels of NT-proBNP in this range signify subclinical cardiac dysfunction of relevance, or other biologies underlying our findings. We do not have cardiac imaging data to further address this.

Strengths and limitations of this study warrant consideration. The prospective nested case control study design allowed us to efficiently address the research question while measuring NT-proBNP in a subsample rather than the entire cohort. The prospective study design minimized the likelihood of reverse causality as an Our rich data set allowed for adjustment for many important measured confounders. Our definition of cognitive impairment captured a clinically relevant level of impairment and had face validity given the previously reported associations of risk factors with impairment [18, 33], however we did not have evaluation for dementia or brain imaging, which are limitations of the study. Follow-up time was relatively short, which could be considered a weakness, but our large sample allowed accumulation of a large number of cases quickly with minimal attrition of participants. Use of the baseline SIS to identify baseline cognitive impairment allowed exclusion of participants with significant impairment but would have missed those with more subtle impairment. This would cause bias in our observed results to the null, thus reported associations might be underestimates. We did not have a measure of cardiac dysfunction such as an echocardiogram. Finally, use of a single measurement of NT-proBNP might lead to underestimation of the true risk of cognitive impairment with higher NT-proBNP.

Results of this study and other research highlight important connections between NT-proBNP and cognitive impairment. Future studies should determine if interventions to improve cardiac function could preserve brain function. Findings also support pursuit of basic research on effects of natriuretic peptides on brain function.

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Table 1. Baseline characteristics of cases and controls.

Baseline Risk Factor Levels % unless otherwise specified	**Cases** **(N= 470)**	**Controls** **(N=557)***	**p-value†**
Black Race	33	36	0.27
Age, Mean	64.5	64.1	0.47
Male Sex	41	43	0.63
Education ≤ High school	34	31	0.43
Stroke Belt Region	66	52	<0.001
Income < $20,000	28	14	<0.001
Diabetes Mellitus	29	18	<0.001
Hypertension	62	56	0.07
Dyslipidemia	60	58	0.46
Atrial Fibrillation	10	9	0.74
Left Ventricular Hypertrophy by ECG	11	7	0.02
History of Heart Disease	21	13	0.003
Congestive Heart Failure	20	13	0.004
Current Smoker	17	12	0.03
Heavy or Moderate Alcohol Use	30	40	0.002
No weekly exercise	38	32	0.05
BMI (kg/m^2), mean	30.1	29.2	0.01
Statin use	34	32	0.42
eGFR <60 ml/min/1.73m^2	14	6	<0.001
Albuminuria, %	18	11	0.006
Apolipoprotein E4 allele present	32	32	0.90

* Data weighted to analytic cohort, total N=17,630

† Pearson chi square (corrected for survey design with the second-order correction of Rao and Scott); Wald F statistic for age and body-mass index.

Table 2. Odds Ratio of Cognitive Impairment by Baseline NT-proBNP

	Quartile of NT-proBNP				
	1	2	3	4	p for
	5-33 pg/ml	33-57 pg/ml	57-127 pgml	>127 pg/ml	trend*
Model 1	1 (Ref)	0.66 (0.43-1.02)	1.30 (0.87-1.94)	1.69 (1.11-2.58)	0.001
Model 2	1 (Ref)	0.73 (0.44-1.21)	1.42 (0.90-2.25)	1.85 (1.14-3.01)	0.002
Model 3	1 (Ref)	0.76 (0.45-1.28)	1.41 (0.88-2.27)	1.67 (1.01-2.77)	0.01
Model 4	1 (Ref)	0.76 (0.46-1.28)	1.50 (0.93-2.42)	1.62 (0.97-2.73)	0.01

*trend across quartiles as an ordinal variable 1, 2, 3, 4.

Model 1. Adjusted for age, sex, race, region, education, income

Model 2. Adjusted for above + hypertension medication use, total and LDL cholesterol, diabetes, systolic blood pressure, left ventricular hypertrophy, alcohol use, physical activity, history of heart disease, atrial fibrillation, and congestive heart failure.

Model 3. Adjusted for above + estimated glomerular filtration rate and urinary albumin/creatinine.

Model 4. Adjusted for above + apolipoprotein e4 allele present

Ref = reference group

Figure Title and Legend

Figure. Association of Baseline NT-proBNP in Quartiles with Risk of Incident Cognitive Impairment by Region. Statistical testing for interaction suggested differences in the association of NT-proBNP with cognitive impairment by region but the figure shows that in stratified analyses these apparent differences had a pattern that was not of material importance. Stroke belt states are North Carolina, South Carolina, Georgia, Tennessee, Mississippi, Alabama, Louisiana, and Arkansas.