Cardiovascular Biomarkers and Subclinical Brain Disease in the Atherosclerosis Risk in Communities Study

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Background and Purpose—Cerebrovascular and cardiovascular disease share common risk factors. Our goal was to determine whether levels of N-terminal brain natriuretic peptide (NT-proBNP) and cardiac troponin T measured with a highly sensitive assay (hs-cTnT) are associated with silent brain infarcts (BIs) and white matter lesions (WMLs) on MRI in the Atherosclerosis Risk in Communities (ARIC) study.

Methods—At ARIC visit 3 (1993–1995), 1920 participants had brain MRI. NT-proBNP and hs-cTnT were measured in all individuals at ARIC visit 4 (1996–1998). Of 1920 individuals, 1112 had a follow-up MRI (2004–2006). We analyzed the association of NT-proBNP and hs-cTnT with MRI-defined BI and WML on the initial MRI and incident BI and WML progression on the follow-up MRI without heart failure, coronary heart disease, or stroke.

Results—In the adjusted model, individuals in the highest NT-proBNP quartile had significantly more BI (odds ratio, 3.50; 95% confidence interval, 2.03–6.20), and WML (β-coefficient, 0.09; SE, 0.03) on the baseline MRI and more incident BI (odds ratio, 2.18; 95% confidence interval, 1.38–3.47) and WML progression (β-coefficient, 0.22; SE, 0.10) on the follow-up MRI. Individuals in the highest hs-cTnT category had more BI (odds ratio, 3.03; 95% confidence interval, 1.57–5.82) and WML progression (β-coefficient, 0.11; SE, 0.04) on the initial MRI and more WML progression (β-coefficient, 0.43; SE, 0.17) on the follow-up MRI.

Conclusions—NT-proBNP and hs-cTnT are independently associated with silent MRI-defined BI and WML, suggesting that cardiovascular biomarkers may be useful to identify individuals with subclinical cerebral injury. (Stroke. 2013;44:00-00.)

Key Words: brain infarcts • white matter lesions • cardiovascular biomarkers • MRI • subclinical brain injury

Cardiac troponin T and N-terminal brain natriuretic peptide (NT-proBNP) are biomarkers currently used in clinical settings in the assessment of chest pain and heart failure (HF)–related symptoms, respectively.1–5 Recent studies in the healthy general population have shown that NT-proBNP and cardiac troponin T levels, measured with a newer highly sensitive assay (hs-cTnT), are associated with incident cardiovascular disease (CVD) events and incident HF hospitalizations.2–5 Thus, hs-cTnT and NT-proBNP are considered to be biomarkers for subclinical cardiac injury in the general population.

It is well-known that cerebrovascular disease and CVD share common risk factors, such as hypertension and diabetes mellitus.6–8 Thus, even before clinical manifestation of organ injury, subclinical brain injury expressed as silent brain infarcts (BIs) and white matter lesions (WMLs) on MRI may be present and coexist with subclinical cardiac injury.9 In healthy middle-aged individuals, the presence of BI and WML on an MRI is associated with higher risk of development of strokes and dementia, which, in turn, result in functional decline, long-term disability, and increased healthcare costs.10–12 Hence, early detection of subclinical brain damage is important. The possible coexistence of subclinical myocardial injury and subclinical brain injury suggests that cardiovascular biomarkers associated with future CVD and HF may also be associated with subclinical brain injury.

The purpose of this study was to investigate the association of hs-cTnT and NT-proBNP with MRI-defined WML and silent BI in the Atherosclerosis Risk in Communities (ARIC) Study.

Original Contribution
Methods

Study Population
The ARIC study is a prospective study of middle-aged predominantly black and white men and women who were 45 to 64 years of age at their baseline examination in 1987–1989. In all, 15,792 participants were sampled from 4 US communities: Forsyth County, NC; Jackson, MS; northwestern suburbs of Minneapolis, MN; and Washington County, MD.

At the third ARIC visit (1993–1995), 2825 persons ≥56 years of age from Forsyth County or Jackson were invited for a brain MRI and 1949 were imaged. Images were of sufficient quality for grading in 2020 examinations. Hs-cTnT and NT-proBNP were measured from the blood samples collected at ARIC visit 4 (1996–1998). After ARIC visit 4, a subset of those individuals who had the initial MRI at ARIC visit 3 (n=1112) had a follow-up MRI between 2004 and 2006 (10.61 years later). For our study, we selected those individuals without a history of CVD or HF who had the initial MRI performed at ARIC visit 3 (Figure I in the online-only Data Supplement). Individuals with missing covariate data (n=283) or those with a history of CVD (coronary heart disease, stroke, or HF; n=135) at the time of hs-cTnT and NT-proBNP measurement were excluded, resulting in 1501 individuals for the NT-proBNP analysis and 1502 individuals for the hs-cTnT analysis. Coronary heart disease and stroke were defined as self-reported myocardial infarction or stroke before ARIC visit 1, or silent myocardial infarction (diagnosed by electrocardiographic changes), validated myocardial infarction, coronary revascularization, or stroke between visits 1 and 4. A detailed description of coronary heart disease events is provided elsewhere.13 HF was identified from hospital discharge records and death certificates that showed an HF code in any position. International Classification of Diseases, Ninth Revision (ICD-9) code 428.x or I50 were considered as HF. Medical history, demographic data, anthropometric data, blood pressure measurements, and fasting lipid assessments were obtained during visit 4 at the same time as the blood draw for hs-cTnT and NT-proBNP measurement. The Institutional Review Board for each ARIC Field Center approved research protocols, and all participants provided written informed consent.

Biomarkers, MRI Scans, and Outcome Definitions
The specific details regarding biomarker assays for hs-cTnT and NT-proBNP have been previously published. The MRI scanning protocol has also been described in detail previously.16 Participants were scanned on 1.5-Tesla scanners. MRIs were interpreted by trained readers at the ARIC MRI Reading Center at Johns Hopkins Medical Institutions in Baltimore.

WMLs were estimated as the total volume of periventricular and subcortical white matter–signal abnormality. WML severity was assessed with a grade from 0 to 9 by visual comparison with 8 templates that successively increased from barely detectable WML to extensive, confluent abnormalities. Subcortical and periventricular WMLs were visually evaluated together. This scale was initially developed and validated in the Cardiovascular Health Study (CHS), and it is described in detail elsewhere.17

BIs were defined by size, type, and location and defined as focal, nonmass areas with an arterial vascular distribution and hyperintense to gray matter on both spin density–weighted and T2-weighted images. BIs in the cerebral white matter and brain stem were defined as lesions with increased signal intensity on spin density–weighted and T2-weighted images and decreased signal intensity on T1-weighted images, similar to the hypointensity of cerebrospinal fluid.18

Statistical Analysis
Our analysis was aimed at answering the following 2 questions: (1) Are higher levels of hs-cTnT and NT-proBNP associated with preexistent subclinical brain injury? (2) Are higher levels of hs-cTnT and NT-proBNP levels associated with incident BI and WML progression? To answer the first question, we analyzed the association of hs-cTnT and NT-proBNP with MRI measures of BI and WML performed at ARIC visit 3 (initial MRI visit). To answer our second question, we analyzed the association of hs-cTnT and NT-proBNP with new BIs and WML progression on the follow-up MRI, which was assessed in comparison with the initial MRI. The follow-up MRI was performed >8 years after measurement of the biomarkers (2004–2006; Figure I in the online-only Data Supplement).

Hs-cTnT and NT-proBNP were modeled as categorical variables. For hs-cTnT, individuals were divided into categories as described in a previous publication1 and are briefly described in the online Data Supplement. For NT-proBNP, quartile measures were used as cut points to obtain 4 groups. The lowest quartile was used as a reference group. The associations between hs-cTnT categories or NT-proBNP quartiles and BI were determined with logistic regression. For analyses of incident BI at the follow-up MRI examination, individuals with MRI-defined BI at the first visit were excluded (n=71). For the association between biomarkers and WML, the β-coefficient of hs-cTnT category or NT-proBNP quartile was obtained from the linear regression analysis modeling log-transformed WML grade [Log(WMG+1)]. Log transformation of WML grade was performed to reduce skewness. Progression in WML burden between the initial and follow-up MRI visits for each individual was calculated as the difference in WML grade between the 2 examinations. The categories and quartiles were modeled as dummy variables, and the β-coefficient represents the difference in log WML grade relative to the first category of hs-cTnT or first quartile of NT-proBNP. In addition, we used linear terms using quartile number or category number to obtain a P value for trend and compare any linear trend in risks with each increasing quartile or category.

The model was adjusted for age, gender, race, ARIC center, hypertension, diabetes mellitus status, smoking status, based on previous results that showed an association between these factors or WML and BI on brain MRIs.12,14 We also adjusted for estimated glomerular filtration rate because of reports that patients with renal insufficiency may have higher cTnT levels and NT-proBNP levels.20,21 There are no data to show an association between low-density lipoprotein cholesterol and high-density lipoprotein cholesterol with small vessel disease of the brain in ARIC.

Results
After exclusion of the individuals with CVD and those with missing covariates, we included in the study a total of 1501 individuals who had an initial MRI performed at ARIC visit 3 and NT-proBNP measured at visit 4. Table I presents the baseline characteristic of each NT-proBNP quartile. The individuals in the highest NT-proBNP quartile were more likely to be women and less likely to be black, and these individuals had lower body mass index. They were also significantly older, more likely to be hypertensive, and less likely to have diabetes mellitus. Individuals in the highest NT-proBNP quartile had higher high-density lipoprotein cholesterol and lower low-density lipoprotein cholesterol levels.

A total of 1502 individuals without CVD had an initial MRI examination at visit 3 and hs-cTnT measured at visit 4. Table 2 presents the baseline characteristics of the individuals by hs-cTnT category. Individuals in the highest category had significantly higher body mass index, were more likely to be black, and less likely to be women. They were significantly older and more likely to be hypertensive and to have diabetes mellitus, and less likely to be smokers or alcohol drinkers. They also had significantly lower levels of high-density lipoprotein cholesterol.

NT-proBNP and MRI Findings
After adjustment for risk factors, when compared with the first quartile, individuals in the highest NT-proBNP...
quartile were more likely to have BIs (odds ratio, 3.50; 95% confidence interval, 2.03–6.20) and more WMLs (β-coefficient, 0.09; SE, 0.03; \( P < 0.01 \)) on the initial MRI (Tables 3 and 4). Of these individuals, 891 had a follow-up MRI 10.61 years later at the follow-up visit (2004–2006). Among these 891 participants, those who were in the highest NT-proBNP quartile had significantly more incident BIs (odds ratio, 2.18; 95% confidence interval, 1.38–3.47) and more WML progression (β-coefficient, 0.22; SE, 0.11; \( P < 0.05 \)) on the follow-up MRI when compared with those in the lowest quartile (Tables 3 and 4).

Hs-cTnT and MRI Findings

When compared with individuals in the lowest category, individuals in the highest hs-cTnT category had significantly more BIs (odds ratio, 3.03; 95% confidence interval, 1.57–5.82) and more WMLs (β-coefficient, 0.11; SE, 0.04; \( P = 0.01 \)) on the initial MRI (Tables 3 and 4). Of these, 892 individuals had a second MRI later at the follow-up visit (2004–2006). Among these, individuals in the highest hs-cTnT category had significantly more WML progression (β-coefficient, 0.43; SE, 0.17; \( P = 0.01 \)) on the follow-up MRI when compared with the lowest category. When compared with the lowest category,
individuals in the higher hs-cTnT categories tended to have more incident BIs (P=0.01), although statistical significance was reached only for the category of hs-cTnT values between 0.009 and 0.013 μg/L (Tables 3 and 4).

Discussion

In the present study, we investigated the association of NT-proBNP and hs-cTnT with silent BIs and WMLs on brain MRI in participants from the ARIC study who were initially free of CVD. Our study shows a positive association between biomarkers of subclinical cardiac injury, such as NT-proBNP and hs-cTnT, and subclinical brain disease diagnosed by the presence of BIs and WMLs on MRI.

Several clinical studies have reported coexistence of heart disease and cerebral disease in elderly individuals.22,23 NT-proBNP and hs-cTnT are both associated with increased risk of incident HF.4,24 A small cross-sectional study by Reinhard et al25 shows that NT-proBNP was positively correlated with WML in patients with diabetes mellitus. Our study extends the findings to a large healthy population-based cohort, showing that patients in the highest quartile of NT-proBNP are more likely to have BIs and have more WMLs on brain MRI. In addition, we show that, prospectively, higher NT-proBNP levels are associated with increased incident BIs and WML progression during ~8 years of follow-up. Further, we show that hs-cTnT levels are associated with silent BIs and WMLs, as well as with WML progression. Previous studies have shown that as many as 20% to 50% of individuals with HF have cognitive impairment,26 but individuals with advanced CVD and symptomatic HF may have cerebral hypoperfusion from reduced cardiac output or subclinical stroke attributable to embolization from a dysfunctional left ventricle or other mechanisms by which advanced CVD could impact the brain directly.

In contrast to these previous studies, we have examined the association of NT-proBNP and hs-cTnT in individuals without any history of HF, coronary heart disease, or stroke. One possible explanation for our findings may be that cerebrovascular disease and cardiac disease share several common risk factors, such as hypertension and diabetes mellitus, which, over time, may produce damage in both organs. When present, these risk factors may promote early changes in the heart that result in alteration in myocardial structure and function, which

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<td>WML progression β (SE)</td>
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Adjusted for age, sex, race, ARIC center, estimated glomerular filtration rate, hypertension, diabetes mellitus, smoking status.

ARIC indicates Atherosclerosis Risk in Communities; BI, brain infarct; CI, confidence interval; NT-proBNP, N-terminal brain natriuretic peptide; OR, odds ratio; and WML, white matter lesions.

*P<0.05 are marked only for the WML. For BI, we present the CIs.

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<th>Table 4. Association of hs-cTnT With MRI Findings</th>
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ARIC indicates Atherosclerosis Risk in Communities; BI, brain infarct; CI, confidence interval; hs-cTnT, cardiac troponin T measured with a highly sensitive assay; OR, odds ratio; and WML, white matter lesions.

*P<0.05 are marked only for the WML. For BI, we present the CIs.
may lead to increased levels of biomarkers such as hs-cTnT and NT-proBNP in blood before clinical symptoms of HF are evident.\textsuperscript{27–29} Eventually, progression of disease leads to development of clinically symptomatic HF. In parallel, these risk factors promote changes in the brain that produce silent BIs and WMLs, which, in time, lead to symptomatic cognitive impairment. In the presence of risk factors, subclinical injury to the brain and heart may occur through mechanisms other than atherosclerosis. Recent studies have shown that arterial stiffness is associated with clinical and subclinical brain disease.\textsuperscript{30–32} At the same time, arterial stiffness was also proven to be associated with NT-proBNP levels in healthy individuals and in individuals with HF.\textsuperscript{33–35} These data are consistent with the hypothesis that arterial stiffness may be a common risk factor for subclinical disease of the heart and brain, which may also explain our findings. Several authors have shown that the pathology of WML and BI reflects arteriosclerosis of the small vessels in the brain.\textsuperscript{7} In hypertensive individuals, smooth muscle cells in the small vessel walls are replaced by fibrohyaline material with thickening of the wall and narrowing of the vascular lumen (arteriosclerosis), resulting in BI and WML.\textsuperscript{6,7} In patients with diabetes mellitus, microvascular disease and lipotoxic mechanisms in the heart may lead to myocardial damage and cTnT leak, whereas in the brain, high blood glucose produces neuronal dysfunction and restriction of axon regeneration, resulting in WML and BI.\textsuperscript{36,37}

One major limitation in our study is that the initial MRI readings performed at ARIC visit 3 were performed 3 years before the troponin and NT-proBNP were measured. Another limitation is the small number of individuals with follow-up MRI performed after ARIC visit 4.

Conclusions

NT-proBNP and hs-cTnT are associated with MRI-defined BIs and WMLs. These cardiovascular biomarkers, which were initially used for diagnosis in the presence of symptoms such as chest pain or shortness of breath, have now been shown to identify individuals who are asymptomatic but at high risk of developing symptomatic CVD.\textsuperscript{3,4} The concept of identifying high-risk individuals with screening blood biomarkers, followed by an imaging test and then therapies directed by a specialist to prevent disease progression, has been recently tested in the cardiovascular field. A study that was recently presented at the American College of Cardiology Scientific Session showed that early measurement of NT-proBNP followed by an echocardiogram for those individuals with values >50 pg/mL and subsequent targeted therapy by a specialist resulted in 46% relative risk reduction in cardiovascular hospitalizations (http://www.theheart.org/article/1518637.do). Perhaps early measurement of the cardiovascular biomarkers NT-proBNP and hs-cTnT followed by an MRI may also help in early detection of cerebrovascular disease before development of symptoms, at a time when interventions directed by a specialist may be beneficial for the prevention of cognitive decline.

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**Biomarker assay**

**Hs-cTnT**

Plasma samples were stored centrally at –80°C and used for measurement of the biomarkers. cTnT levels were measured by using a novel precommercial highly sensitive assay, Elecsys Troponin T (Roche Diagnostics, Indianapolis, IN), on an automated Cobas e411 analyzer with a lower limit of detection of 0.003 µg/L. The between-assay coefficient of variation was 2.6% and 6.9% for control materials with mean cTnT concentrations of 2.378 µg/L and 0.029 µg/L, respectively (approximately the 99th percentile of ARIC). Repeatability of measurements was assessed by using blinded split samples (n=418). The reliability coefficient was 0.98, and coefficient of measurement variation was 15% when excluding >3-standard deviation outliers (n=3). Assays on repeat samples drawn within 2–6 weeks also showed high reliability coefficients.

**NT-proBNP**

Serum NT-proBNP concentration was determined using Elecsys proBNP sandwich immunoassay on an Elecsys 2010 (Roche Diagnostics). The analytical range was 5.1–34927 pg/mL. Between-assay coefficients of variation in low and high ranges of NT-proBNP are reported to be 4.8 and 2.7% respectively.

**Follow up MRI scanning (2004-2006).**

All participants who had the initial MRI were invited forth repeat MRI however only 1112 had the repeat MRI. The rest of the participants (n=808) did not undergo repeat scanning for different reasons (death (n = 268), refusal (n = 452), requests for no further contact with study (n = 20), neurologic disorders (n = 13), surgery/radiation to skull/brain (n = 9), or ineligible (n = 46)).
Statistical analysis

Hs-cTnT categories: The 29% individuals with undetectable levels were the reference group (category 1). The remaining 71% were split into approximate thirds: hs-cTnT levels 0.003 to 0.005 µg/L (category 2), 0.006 to 0.008 µg/L (category 3), and higher levels divided at approximately the 90th percentile of the ARIC population (category 4: 0.009 to 0.013 µg/L; category 5: ≥0.014 µg/L), which incidentally corresponded to the 99th percentile value specified by the manufacturer.
Figure legend 1: The average time between First MRI at Visit 3 and the time of biomarker measurement was 3.01 years. The average time between the two MRIs was 10.61 years. In order to answer question 1 we analyzed individuals free of CVD who had biomarkers measured at visit 4 and first MRI performed (1514 for the NT-proBNP and 1515 for hs-cTnT). In order to answer question 2 we analyzed subjects who had the follow-up MRI and biomarkers measured at visit 4 (891 for the NT-proBNP and 892 patient for the troponin analysis).