Circulating Levels of Biomarkers of Cerebral Injury in Patients with Atrial Fibrillation

Oxana Galenko, DBMSc[a], Victoria Jacobs, PhD[a], Stacey Knight, PhD[a,b], Daniel Bride, M.S[a], Michael J. Cutler, DO, PhD[a], Joseph B. Muhlestein, MD[a,b], John L. Carlquist, PhD[a], Jeffrey L. Anderson, MD[a,b], Kirk U. Knowlton, MD[a,b], and T. Jared Bunch, MD, FHRS[a,c,*]

Atrial fibrillation (AF) is a source of altered brain perfusion and ischemia, potentially leading to cerebral injury and blood brain barrier (BBB) disruption, which may result in the permeation of neurospecific molecules into the bloodstream. We retrospectively analyzed circulating levels of biomarkers of cerebral injury: Astrocyte-specific glial acidic fibrillary protein (GFAP), calcium-binding protein B (S100 b), stress response marker growth differential factor 15 (GDF15), and microtubule associated Tau protein, in patients with AF and non-AF controls. A total of 196 AF cases and 47 non-AF controls were enrolled in this study all without previous clinical stroke or cerebral injury. Plasma samples were obtained from the Intermountain INSPIRE biobank registry. AF status was determined at the time of the sample draw using clinical diagnosis. Assessment of circulating biomarkers was conducted with EIA. Multivariate linear modeling, using natural log, and square root transformation of the biomarkers, was done adjusting for (1) CHA_2DS_2-VASc and anticoagulation, and (2) age, gender, coronary artery disease and anticoagulation. Circulating Tau, GDF15, and GFAP were elevated in AF cases. After multivariate adjustment, GFAP and Tau remained significantly elevated in the AF, whereas the signal for GDF15 was confounded by age. In conclusion, circulating biomarkers of neuronal and glial injury Tau and GFAP are elevated in patients with AF that are consistent with subclinical cerebral injury and disruption of the BBB, which can predispose these patients to the development of cognitive dysfunction and/or dementia later in life. **(Am J Cardiol 2019;124:1697−1700)**

Atrial fibrillation (AF) is the most common arrhythmia encountered in the clinical setting.[1] AF has been associated with multiple adverse comorbidities including the development of dementia in patients without previous stroke.[2-5] It has been estimated that 35.6 million people worldwide were diagnosed with dementia in 2010 and to increase to 100 million people by 2050.[6] AF is an independent risk factor for the development of dementia and each disease shares similar risk factors.[7] Various models have been proposed to explain the association of AF and dementia, such as: fluctuations in brain blood perfusion during the episodes of arrhythmia; cerebral microemboli and microbleeds, and inflammatory and prothrombotic processes.[8,9] All of the above, or a combination of these mechanisms, can cause cerebral injury with blood brain barrier (BBB). Our approach to cerebral injury in patients with AF remains limited due to an incomplete understanding of these mechanisms and is primarily focused on reducing a risk of a disabling stroke. In this study we retrospectively analyzed circulating levels of biomarkers of cerebral injury: astrocyte-specific glial acidic fibrillary protein (GFAP), calcium-binding protein B (S100 b), stress response marker growth differential factor 15 (GDF15), and microtubule associated Tau protein, in patients with and without AF to determine if these neuron-specific biomarkers of injury are elevated in patients with AF.

Methods

We studied 243 subjects (196 AF cases and 47 non-AF controls) from the Intermountain INSPIRE biobank registry. This registry was approved by the Institutional Review Board and patients were included after giving informed consent. AF cases were diagnosed with AF before the blood collection and were selected using diagnoses codes (ICD-9: 427.31, and ICD-10: I48.0, I48.1, I48.2, I48.91) and did not have to be in AF at the time of their blood draw. The control patients had no diagnostic codes for AF nor evidence of AF in our electrocardiogram and ambulatory monitor databases. Both cases/control statuses were further confirmed by manual chart review. Patients with a history of stroke and cognitive dysfunction before the blood draw were excluded from the study. The plasma from these subjects was processed and banked under standard conditions. Plasma samples were stored at −80 C° until tests were performed. Assessment of circulating biomarkers was conducted with enzyme-linked immunosorbent assay (My BioSource Inc and R&D Systems Inc). The distributions of the biomarkers were skewed, and

[a]Intermountain Medical Center Heart Institute, Intermountain Medical Center, Salt Lake City, Utah; [b]Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, Utah; and [c]Stanford University, Department of Internal Medicine, Stanford, California. Manuscript received June 3, 2019; revised manuscript received and accepted August 19, 2019.

Funding: This research was funded by a grant from the Intermountain Medical Research Foundation, Salt Lake City, Utah.

See page 1700 for disclosure information.

*Corresponding author: Tel: (801) 507-3513; fax: (801) 507-3584.

E-mail address: Thomas.bunch@imail.org (T. Jared Bunch).

0002-9149/
https://doi.org/10.1016/j.amjcard.2019.08.027

thus they were natural log (Tau, GFAP, GDF15) and square root (S100B) transformed before modeling linear relationships using linear regression. Results for the biomarkers were compared by AF status. The linear modeling was done adjusting for 2 sets of variables first, by CHA_2DS_2-VASc score and anticoagulant medication use; and second, by age, gender, previous coronary artery disease, and anticoagulant medication use.

Results

Patients' baseline characteristics at the time of plasma acquisition are summarized in Table 1. The AF cases were significantly older (67 ± 11 years old) when compared with non-AF controls (58 ± 12 years old) ($p < 0.0001$). The AF cases also had a higher rate of coronary artery disease (16.3%) when compared with non-AF controls (3.0%) ($p = 0.008$). The AF patients were more likely to be prescribed cardiovascular medications, especially anticoagulants (71.9% vs 8.5%; $p < 0.001$). The AF cases had higher CHA_2DS_2-VASc scores than the controls with 73.5% having a CHA_2DS_2-VAS score ≥2 compared with 57.4% for non-AF controls ($p = 0.02$).

Circulating biomarkers of cerebral injury were higher in AF cases than non-AF controls for Tau, GFAP, and GDF15 but not S100B (Figure 1). After multivariate adjustment for CHA_2DS_2-VASc and anticoagulation, AF was associated with higher levels of Tau, GFAP, and GDF15 ($p = 0.008$, $p < 0.0001$ and $p = 0.01$, respectively) but not S100B ($p = 0.41$). Although the second set of adjustment for age, gender, previous CAD, and anticoagulation found similar significant associations between AF and Tau ($p = 0.009$) and GFAP ($p < 0.0001$), the association between AF and GDF15 became nonsignificant ($p = 0.13$), as age was confounding the previous significant signal. We performed both untransformed and the natural log transformation to further examine the association of age with the biomarkers. GDF15 ($p < 0.0001$, $p < 0.0001$) was associated with aging, whereas Tau ($p = 0.15$, $p = 0.28$), GFAP ($p = 0.12$, $p = 0.004$), and S100B ($p = 0.21$, $p = 0.22$) were not or to a lesser degree associated by aging, by untransformed and natural log transformation, respectively.

Table 1
Baseline characteristic comparisons between AF cases and controls

Variables	Atrial fibrillation		p Value*
	YES (n = 196)	NO (n = 47)	
Age	66.8 ± 10.7	57.8 ± 11.8	<0.0001
Men	118 (60%)	21 (45%)	0.05
White	175 (91%)	43 (92%)	0.88
Smoker	8 (4%)	8 (17%)	0.004
Hypertension	111 (57%)	30 (64%)	0.37
Hyperlipidemia	76 (39%)	24 (51%)	0.12
Diabetes mellitus	48 (25%)	14 (30%)	0.45
Prior myocardial infarction	9 (5%)	0 (0%)	0.21
Prior coronary artery disease	32 (16%)	1 (3%)	0.008
Prior heart failure	41 (21%)	6 (13%)	0.20
Medications			
Statins	71 (36%)	4 (9%)	0.0002
Aspirin	128 (67%)	12 (9%)	<0.0001
Other antiplatelet	17 (9%)	0 (0%)	0.03
Anticoagulant	138 (72%)	4 (9%)	<0.0001
Aspirin and anticoagulant	97 (49%)	3 (6.4%)	0.0001
Aspirin and antiplatelet	10 (5%)	0 (0%)	0.22
Beta blocker	63 (33%)	5 (11%)	0.003
Calcium channel blocker	41 (21%)	1 (2%)	0.002
Diuretic	62 (32%)	5 (11%)	0.003
Angiotensin-converting enzyme inhibitors	54 (28%)	5 (11%)	0.01
Angiotensin II receptor blockers	28 (15%)	1 (2%)	0.02
CHA_2DS_2-VASC			0.02
0	7 (4%)	6 (13%)	
1	45 (23%)	14 (30%)	
≥2	144 (74%)	27 (57%)	

* p values from univariate chi-square and Wilcoxon rank sum tests.

Discussion

This study has demonstrated that patients with AF without a previous history of cerebral injury, stroke, cognitive decline or dementia have elevated levels of the Tau protein, GDF15 and GFAP in the systemic circulation, which is indicative of neuronal and glial damage with probable disruption of the BBB. These new findings have potentially significant implications related to the early detection of neurological injury in AF patients.[10–12]

Tau is a microtubule associated protein that is specifically expressed in axons of cortical interneurons, and which plays an important role in microtubule assembly, neuronal development, and axonal stability.[13] Variations in cerebral perfusion, along with specific proinflammatory states alter the BBB, increasing its permeability in AF patients. Extracellular release of Tau due to neuronal injury from affected axons and increased BBB permeability renders it detectable in the systemic circulation at a higher concentration level as seen with other forms of repetitive cerebral or cranial injury.[14]

GFAP is predominantly a brain-specific protein, expressed in high quantities in astrocytes of brain tissue. Incidents involving glial injury cause GFAP to be released into the extracellular space and then to circulating blood, either by direct venous drainage or by diffusion through a compromised BBB.[15] Elevated circulating levels of GFAP have been detected in patients with severe traumatic brain injury and were predictive of acute and long-term unfavorable neurological outcomes. In our study, elevation of GFAP in plasma suggests that AF patients experience astroglial damage and BBB dysfunction.

Surprisingly, S100B was not significantly elevated in AF patients. S100B is an intracellular astrocyte-and oligodendrocyte-specific protein. It was recently demonstrated that S100B is also present in adipocytes and chondrocytes.[16] An elevated level of circulating S100B is also a marker for neurological stress or BBB disruption; however, there are reports that S100B might be lacking in specificity and sensitivity due to its extracerebral presence.[17]

GDF15 is a member of the TGF-β superfamily, and is a proven marker of oxidative stress and inflammation, and is a risk predictive marker for bleeding and all-cause mortality in AF patients.[18] GDF15 is expressed in various tissues, is elevated in response to brain injury, and also is associated with both cognitive decline and Alzheimer's dementia (AD) when significant white matter hyperintensities are detected.[19,20] In

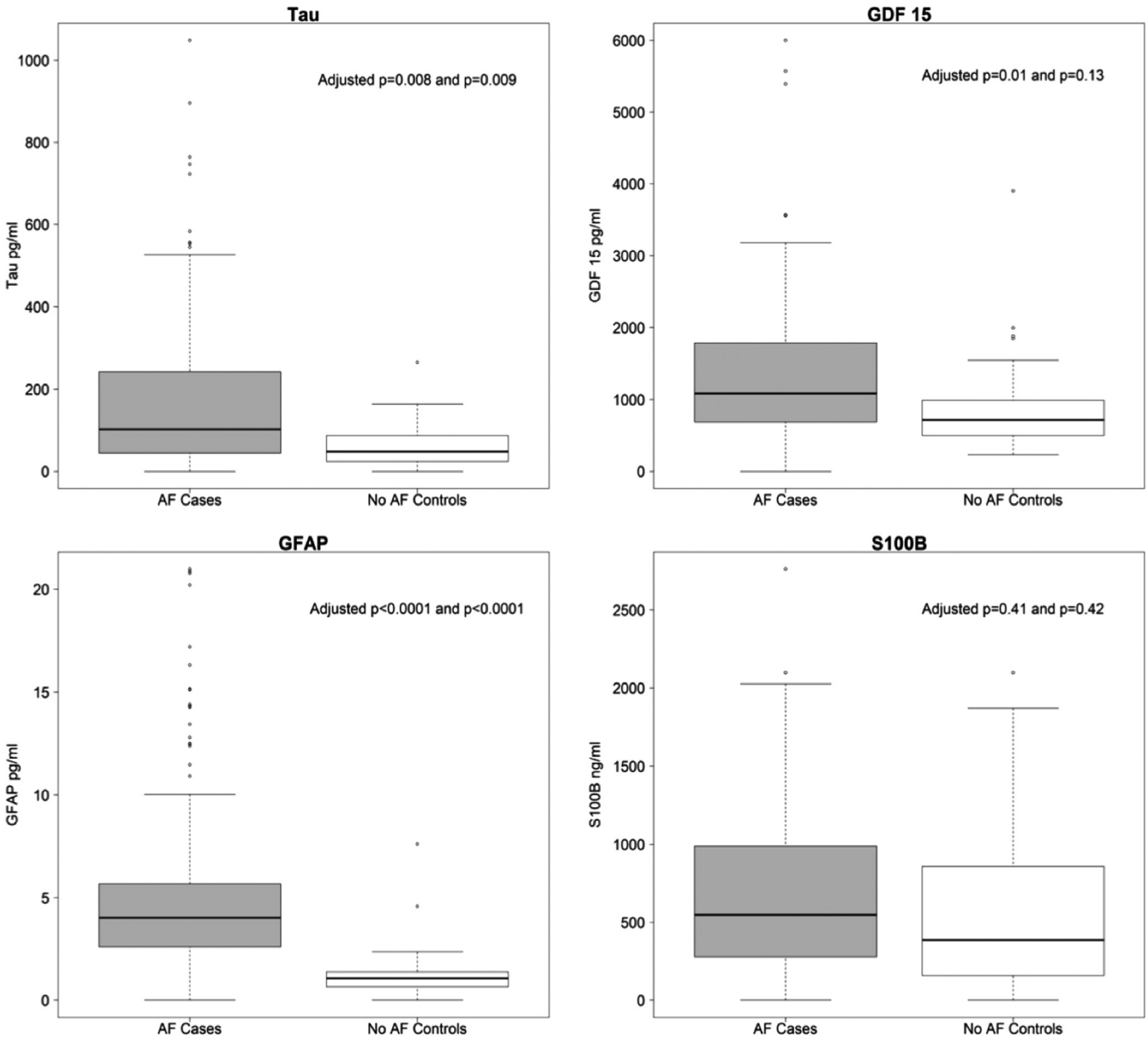

Figure 1. Biomarker distributions of AF (cases) versus non-AF patients (controls). p values adjusted for (1) CHA_2DS_2-VASc and anticoagulation medication, and (2) age, gender, previous CAD, and anticoagulant medication.

our study, GDF15 was significantly increased in AF patients only in univariate analyses and GDF15 demonstrated a strong age-based association of elevation.

Based on our findings, cerebral injury in AF patients likely develops early in the disease process before clinical manifestations are present. These injuries in susceptible patients over time may reach a tipping point when cerebral dysfunction becomes detectable from a clinical standpoint. This hypothesis is supported by a recent report that 41% of patients with AF, with no history of previous stroke or transient ischemic attack, have otherwise asymptomatic brain lesions detected on MRI.[21,22] Early detection of cerebral injury is essential for taking preventive measures and further understanding of potential mechanisms before the clinical manifestations of cognitive impairment appear. Prospective evaluation of these markers, as well as others, is ongoing as part of the Concussion Atrial Fibrillation trial (clinicaltrials.gov NCT03875131) that will also provide clinical correlation.

This study is subject to several limitations, including those inherent to all retrospective case-control designs. First, it was conducted on a relatively small sized control group which did not allow us to perform case-control matching to eliminate baseline differences. These controls also were patients undergoing assessment by a cardiologist and, therefore, not randomly selected healthy controls. However, we suspect that using healthy control might provide an even stronger signal. Although none of the patients had a previous stroke or significant cranial injury, we cannot account for other potential neurological injuries. However, we believe these would be rare and should not be associated with the case status. Another limitation is that the cases and controls had significant baseline differences,

in particular age. Although we attempted to adjust for this in the multivariable analyses as well as untransformed and natural log transformation, the statistical adjustment might not have fully accounted for possible confounding effects.

In conclusion, biomarkers of neuronal and glial damage are elevated in patients with AF that are consistent with subclinical cerebral injury and disruption of the BBB. Specifically, levels of circulating Tau and GFAP were found to be higher in AF patients and suggest detectable and potential repetitive brain injury, which can predispose these patients to the development of cognitive dysfunction and/or dementia later in life.

Disclosures

The authors have no conflicts of interest to disclose.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2019.08.027.

1. Chinitz JS, Castellano JM, Kovacic JC, Fuster V. Atrial fibrillation, stroke, and quality of life. *Ann N Y Acad Sci* 2012;1254:140–150.
2. Santangeli P, Di Biase L, Bai R, Mohanty S, Pump A, Brantes MC, Horton R, Burkhardt JD, Lakkireddy D, Reddy YM. Atrial fibrillation and the risk of incident dementia: a meta-analysis. *Heart Rhythm* 2012;9:1761–1768.
3. Charidimou A, Inamura S, Nomura T, Kanno A, Kim SN, Imaizumi T. Cerebral microbleeds and white matter hyperintensities in cardioembolic stroke patients due to atrial fibrillation: single-centre longitudinal study. *J Neurol Sci* 2016;369:263–267.
4. Charidimou A, Shoamanesh A, Wilson D, Gang Q, Fox Z, Jager HR, Benavente OR, Werring DJ. Cerebral microbleeds and post-thrombolysis intracerebral hemorrhage risk: updated meta-analysis. *Neurology* 2015;85. 15927-924.
5. Kwok CS, Loke YK, Hale R, Potter JF, Myint PK. Atrial fibrillation and incidence of dementia: a systematic review and meta-analysis. *Neurology* 2011;76:914–2226.
6. Prince M, Bryce R, Albanese E, Wimo A, Ribeiro W, Ferri CP. The global prevalence of dementia: a systematic review and metaanalysis. *Alzheimers Dement* 2013;9:63–75.
7. Bunch TJ, Weiss JP, Crandall BG, May HT, Bair TL, Osborn JS, Anderson JL, Muhlestein JB, Horne BD, Lappe DL, Day JD. Atrial fibrillation is independently associated with senile, vascular, and Alzheimer's dementia. *Heart Rhythm* 2010;7:433–437.
8. Jacobs V, Woller SC, Stevens S, May HT, Bair TL, Anderson JL, Crandall BG, Day JD, Johanning K, Long Y. Time outside of therapeutic range in atrial fibrillation patients is associated with long-term risk of dementia. *Heart Rhythm* 2014;11:2206–2213.
9. Graves KG, May HT, Jacobs V, Bair TL, Stevens SM, Woller SC, Crandall BG, Cutler MJ, Day JD, Mallender C, Osborn JS, Peter Weiss J, Jared Bunch T. Atrial fibrillation incrementally increases dementia risk across all CHADS2 and CHA2DS2VASc strata in patients receiving long-term warfarin. *Am Heart J* 2017;188:93–98.
10. Jacobs V, Cutler MJ, Day JD, Bunch TJ. Atrial fibrillation and dementia. *Trends Cardiovasc Med* 2015;25:44–51.
11. Kalantarian S, Ay H, Gollub RL, Lee H, Retzepi K, Mansour M, Ruskin JN. Association between atrial fibrillation and silent cerebral infarctions: a systematic review and meta-analysis. *Ann Intern Med* 2014;161:650–658.
12. Kalantarian S, Stern TA, Mansour M, Ruskin JN. Cognitive impairment associated with atrial fibrillation: a meta-analysis. *Ann Intern Med* 2013;158(5 Pt 1):338–346.
13. Trojanowski JQ, Schuck T, Schmidt ML, Lee VM. Distribution of tau proteins in the normal human central and peripheral nervous system. *J Histochem Cytochem* 1989;37:209–215.
14. Shahim P, Tegner Y, Wilson DH, Randall J, Skillback T, Pazooki D, Kallberg B, Blennow K, Zetterberg H. Blood biomarkers for brain injury in concussed professional ice hockey players. *JAMA Neurol* 2014;71:684–692.
15. Yang Z, Wang KK. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. *Trends Neurosci* 2015;38:364–374.
16. Olsson B, Zetterberg H, Hampel H, Blennow K. Biomarker-based dissection of neurodegenerative diseases. *Prog Neurobiol* 2011;95:520–534.
17. Marchi N, Rasmussen P, Kapural M, Fazio V, Kight K, Mayberg MR, Kanner A, Ayumar B, Albensi B, Cavaglia M, Janigro D. Peripheral markers of brain damage and blood-brain barrier dysfunction. *Restor Neurol Neurosci* 2003;21(3-4):109–121.
18. Hijazi Z, Oldgren J, Andersson U, Connolly SJ, Eikelboom JW, Ezekowitz MD, Reilly PA, Yusuf S, Siegbahn A, Wallentin L. Growth-differentiation factor 15 and risk of major bleeding in atrial fibrillation: insights from the randomized evaluation of long-term anticoagulation therapy (RE-LY) trial. *Am Heart J* 2017;190:94–103.
19. Schober A, Bottner M, Strelau J, Kinscherf R, Bonaterra GA, Barth M, Schilling L, Fairlie WD, Breit SN, Unsicker K. Expression of growth differentiation factor-15/ macrophage inhibitory cytokine-1 (GDF-15/ MIC-1) in the perinatal, adult, and injured rat brain. *J Comp Neurol* 2001;439:32–45.
20. Fuchs T, Trollor JN, Crawford J, Brown DA, Baune BT, Samaras K, Campbell L, Breit SN, Brodaty H, Sachdev P, Smith E. Macrophage inhibitory cytokine-1 is associated with cognitive impairment and predicts cognitive decline - the Sydney Memory and Aging Study. *Aging cell* 2013;12:882–889.
21. Chai YL, Hilal S, Chong JP, Ng YX, Liew OW, Xu X, Ikram MK, Venketasubramanian N, Richards AM, Lai MK, Chen CP. Growth differentiation factor-15 and white matter hyperintensities in cognitive impairment and dementia. *Medicine* 2016;95:e4566.
22. Blum S, Kuehne M, Rodondi N, Mueller A, Ammann P, Moschovitis G, Kobza R, Schlaepfer J, Meyre P, Bonati L. 1358 prevalence of silent vascular brain lesions among patients with atrial fibrillation and no known history of stroke. *Eur Heart J* 2018;39(suppl_1). ehy565. 1358.