Investigating the Contributions of Blood-Brain Barrier Dysfunction to the Risk of Cognitive Decline and Dementia in Individuals with Atrial Fibrillation

Danielle Lee Marion

Thesis Supervisor:
Dr. Jodi D. Edwards, PhD

Thesis Advisory Committee Members:
Dr. Ellen E. Freeman, PhD
Dr. Shawn N. Whitehead, PhD

Thesis submitted to the University of Ottawa
in partial fulfillment of the requirements for the
Master of Science degree in Epidemiology

School of Epidemiology and Public Health
Faculty of Medicine
University of Ottawa

Preface to the Thesis

We obtained data use approvals and executed data sharing agreements with the Reasons for Geographic and Racial Differences in Stroke study and the Cardiovascular Health Study committees to conduct this research. Ethics approval was granted by the University of Ottawa Heart Institute Research Ethics Board. Danielle Marion was responsible for the analyses, the interpretation of the results, and the writing of the thesis and included manuscripts. Dr. Jodi Edwards was responsible for the supervision of the thesis research and contributed to the study conception, data access, analyses and interpretation, and revision of the thesis. As members of the thesis committee, Dr. Ellen Freeman and Dr. Shawn Whitehead provided input on the thesis questions, guidance on the conduct of the research, and revised the thesis. For the thesis manuscripts, Dr. Zhe Li provided analytic support for the latent growth curve analyses, Austyn Roseborough provided expertise in blood-brain barrier dysfunction and neuroinflammation, Dr. David Birnie provided clinical expertise, and Dr. Fanny Elahi provided expertise in biomarkers of neurodegenerative disease. Dr. Vladimir Hachinski contributed to the generation of the research hypothesis.

Abstract

Despite evidence for an association between atrial fibrillation (AF) and cognitive decline and dementia independent of stroke, pathways underlying this relationship remain unclear. Critically, elevated levels of inflammatory markers, common in AF, are associated with the breakdown of the blood-brain barrier (BBB) and may contribute to neuroinflammation and neurodegeneration. To investigate this potential contributing pathway, we estimated associations of inflammatory markers with cognitive decline and dementia in AF adults. We used data from two population-based cohorts and found that inflammatory markers were associated with cognitive decline but not

dementia. Some associations were modified by sex and apolipoprotein E (APOE) genotype. These findings provide preliminary evidence for inflammatory-mediated BBB dysfunction as a potential contributing pathway linking AF to cognitive decline. Future work examining the role of BBB dysfunction in AF and cognition may benefit from the use of markers of central inflammation to increase sensitivity, while considering possible differences by sex, dementia subtype, and APOE genotype.

Acknowledgments

This thesis would have not been possible without the help and support of many people. First and foremost, I would like to thank my supervisor, Dr. Jodi Edwards, for her guidance and insightful feedback and whose enthusiasm and encouragement inspired me. I am extremely grateful to have had an outstanding mentor whose expertise was invaluable in shaping this thesis as well as my critical thinking skills. I would like to extend my deepest appreciation to my committee members, Dr. Ellen Freeman and Dr. Shawn Whitehead, for their oversight of this research and their valuable suggestions. Thank you to the co-authors (Dr. Zhe Li, Austyn Roseborough, Dr. David Birnie, Dr. Fanny Elahi, and Dr. Vladimir Hachinski) who have greatly improved the included manuscripts with their extensive knowledge. I would also like to acknowledge that this research was funded by a grant from the Canadian Institutes of Health Research (PJT 169192).

Finally, I would like to thank my family for their unwavering love and support. To my parents, thank you for providing me with countless opportunities throughout my life and for instilling in me the importance of dedication. You have played an instrumental role in helping me achieve my goals. To my loving and supportive wife, Julia, thank you for your endless patience and for being my sounding board. I am eternally grateful for you.

Table of Contents

List of Tables

Chapter 4

Chapter 5

List of Figures

Chapter 2

Chapter 4

Chapter 5

Appendix

List of Abbreviations

Aβ	Amyloid beta
AD	Alzheimer's disease
AF	Atrial fibrillation
AIC	Akaike information criterion
ANOVA	Analysis of variance
APOE	Apolipoprotein E
BBB	Blood-brain barrier
BIC	Bayesian information criterion
BMI	Body mass index
$CHADS_2$	Congestive heart failure, hypertension, age ≥ 75 years, diabetes, stroke
CHA_2DS_2-VASc	Congestive heart failure, hypertension, age ≥ 75 years, diabetes mellitus, stroke or transient ischemic attack (TIA), vascular disease, age 65 to 74 years, sex category
CHCS	Cardiovascular Health Cognition Study
CHS	Cardiovascular Health Study
CI	Confidence interval
CNS	Central nervous system

CRP	C-reactive protein
CVD	Cardiovascular disease
ECG	Electrocardiogram
EV	Extracellular vesicles
HR	Hazard ratio
IL-6	Interleukin-6
IQ CODE	Informant questionnaire for cognitive decline in the elderly
LCGA	Latent class growth analysis
LVH	Left ventricular hypertrophy
MCI	Mild cognitive impairment
OR	Odds ratio
REGARDS	Reasons for Geographic and Racial Differences in Stroke
ROS	Reactive oxygen species
RR	Relative risk
SCI	Silent cerebral infarction
SIS	Six-item screener
TARMOS	Treatment and reporting of missing data in observational studies
TIA	Transient ischemic attack

TICS	Telephone interview for cognitive status
VaD	Vascular dementia
WMG	White matter grade
3MS	Modified Mini-Mental State Examination

Chapter 1. Introduction

1.1 Overview

Atrial fibrillation (AF) and dementia are growing public health burdens in the aging population. AF, the most common type of cardiac arrhythmia, is associated with an increased risk of morbidity and mortality, increasing stroke risk 5-fold (1). Future projections suggest that the global prevalence of AF will be more than 60 million by 2050 (2). Dementia is a leading chronic disease contributing to disability and dependence among older adults (3). With the advancing age of the population, the number of people living with dementia is expected to rise to 139 million by 2050, presenting a substantial public health concern (4). As stroke increases the risk of dementia 2-fold (5), stroke can act as an intermediate between AF and dementia. Critically, evidence shows that AF patients are at a higher risk of cognitive decline and dementia, independent of stroke (6). Despite strong evidence supporting this association, the underlying pathways linking AF to dementia remain poorly understood.

A potential contributing pathway of this association that has received relatively little attention is the pro-inflammatory state common to both AF and dementia (7) and its impact on the blood brain barrier (BBB). Inflammation has been associated with the initiation and perpetuation of AF and with neurodegenerative changes (8–11). Blood vessels that vascularize the central nervous system (CNS) have specialized structural and functional characteristics that collectively form the BBB, which separates the peripheral circulation from the brain parenchyma (12). Systemic inflammation in AF may cause the breakdown of the BBB, which has been strongly related to dementia and cognitive impairment (12). As the BBB normally shields the brain from toxic substances in the blood, its breakdown subjects the brain to neurotoxins, which activates central inflammatory responses and impairs the clearance of amyloid beta (Aβ) leading to its

accumulation and aggregation in the brain (12,13). This results in neurodegeneration, synaptic impairment, and neuronal damage leading to cognitive decline and dementia (12). Elevated plasma levels of interleukin-6 (IL-6), C-reactive protein (CRP), and fibrinogen (markers of systemic inflammation) are associated separately with AF and BBB breakdown (14–20). However, the extent to which inflammatory-mediated BBB dysfunction contributes as a pathway of cognitive decline and dementia in individuals with AF remains unknown. This thesis will explore this question.

1.2 Rationale

Dementia is a growing public health concern (4). As treatment trials testing amyloid-lowering therapies have been largely ineffective, there are currently no approved disease-modifying drugs available to prevent or slow the progression of dementia (21). There is thus an urgent need to better understand the pathophysiology of the disease and differentiate vascular from neurodegenerative pathways to identify new potential treatment targets. Given dementia's long preclinical phase, risk reduction and early intervention are critical to prevent or delay the progression of this disabling disease. This is particularly true for the AF population, who have an increased risk of cognitive decline and dementia, independent of stroke (6) and for whom the vascular contributions to cognitive decline have the potential to be mitigated by the early initiation of oral anticoagulation therapy (22). Given the increasing prevalence of AF and significant impact of dementia on patients, carers and the health system, a more detailed understanding of the pathways underlying the association between AF and dementia is required for the early identification and treatment of cognitive risk. This master's thesis addresses critical gaps in our knowledge and explores inflammatory-mediated BBB dysfunction as a potential pathway of the vascular contributions to cognitive decline in the AF population. Reducing inflammation and maintaining the integrity of

the BBB may be an important treatment target for patients with AF for early prevention of cognitive decline and dementia. There is also a critical need for the identification of biomarkers of BBB integrity that are predictive of cognitive decline and dementia. Blood-based measures of plasma IL-6, CRP, and fibrinogen may fill this critical gap and improve risk prediction tools for dementia to guide treatment decisions in the AF population.

1.3 Research Objectives

The overall objective of this research is to examine inflammatory-mediated BBB dysfunction as a potential contributing pathway of cognitive decline and dementia in individuals with AF. To directly address these knowledge gaps, the following research objectives were addressed:

1. Estimate associations between plasma-based markers of inflammation (including fibrinogen, CRP, and IL-6) and incident dementia in aging adults with documented AF.
2. Estimate associations between plasma-based markers of inflammation (including fibrinogen, CRP, and IL-6) and cognitive decline and worsening white matter grade on MRI in aging adults with documented AF.
3. Investigate these associations within subgroups of interest: by sex and apolipoprotein (APOE) genotype.

1.4 Thesis Structure

This article-based thesis has 6 chapters. Chapter 1 introduces the topic, the rationale for the thesis, and the specific research objectives. Chapter 2 presents a review of the literature on AF and dementia, evidence from population-based studies of the association between AF and dementia, the proposed pathways behind this association (focusing on the pro-inflammatory hypothesis leading to BBB dysfunction), and an outline of the conceptual framework for the studies. Chapter 3 expands on important methodological and analytical aspects excluded in the manuscripts and

Chapters 4 and 5 present the first and second manuscripts, respectively. Lastly, Chapter 6 presents a discussion and conclusion, which provides an overall interpretation of the two manuscripts, the strengths and limitations, and implications for future research and practice.

Chapter 2. Literature Review

2.1 Atrial Fibrillation

2.1.1 Definition, burden, and risk factors

AF is the most common sustained cardiac arrhythmia, with a global prevalence of over 37 million (2). In AF, uncoordinated electrical activation of the upper chambers of the heart, called the atria, causes an irregular, often rapid, heartbeat (23). Advancing age is the most important risk factor for AF (24). Studies have consistently found that the prevalence and incidence of AF increases with age, particularly among those 65 years or older (25,26). Often the increasing burden of comorbidities and cardiovascular risk factors contribute to the development and/or progression of AF (27). In particular, hypertension, male sex (25,26,28), diabetes mellitus, heart failure, and valvular heart disease are well established risk factors for AF (29,30). Future projections suggest that more than 60 million individuals will have AF by 2050, owing to the aging population and improved survival with cardiovascular conditions (2,24).

2.1.2 Mechanisms

AF can result from ectopic foci that initiate electrical impulses from locations outside the normal conduction route and/or a re-entry circuit, the repetitive propagation of an impulse in a circular path (31) (see Figure 2-1 below). The initiation and maintenance of AF can be linked to the interaction between a trigger and the substrate (32) (see Figure 2-2). The trigger, a rapidly firing focus, acts as the initiator of AF, whereas the substrate, electrical and structural remodelling of the

atria, is required to sustain the arrhythmia (32). Electrical remodelling includes changes to ion channel function necessary for proper activation and conduction, whereas structural remodelling includes alterations to atrial tissue architecture, such as atrial fibrosis or dilation (32). AF often initially presents as intermittent or paroxysmal, which is mostly trigger-driven (32). It is thought that the arrhythmia progresses to a persistent or permanent form through the additional influence of atrial remodelling (31,32).

Figure 2-1. Principal mechanisms of atrial fibrillation (AF). A. Local ectopic firing. **B.** Single-circuit re-entry. **C.** Multiple-circuit re-entry. **D.** Clinical AF forms and relation to mechanisms. Paroxysmal forms show a predominance of local triggers/drivers, particularly from pulmonary veins (PVs). As AF becomes more persistent and eventually permanent, re-entry substrates (initially functional and then structural) predominate. RA indicates right atrium; SVC, superior vena cava; LA, left atrium; and IVC, inferior vena cava (31).

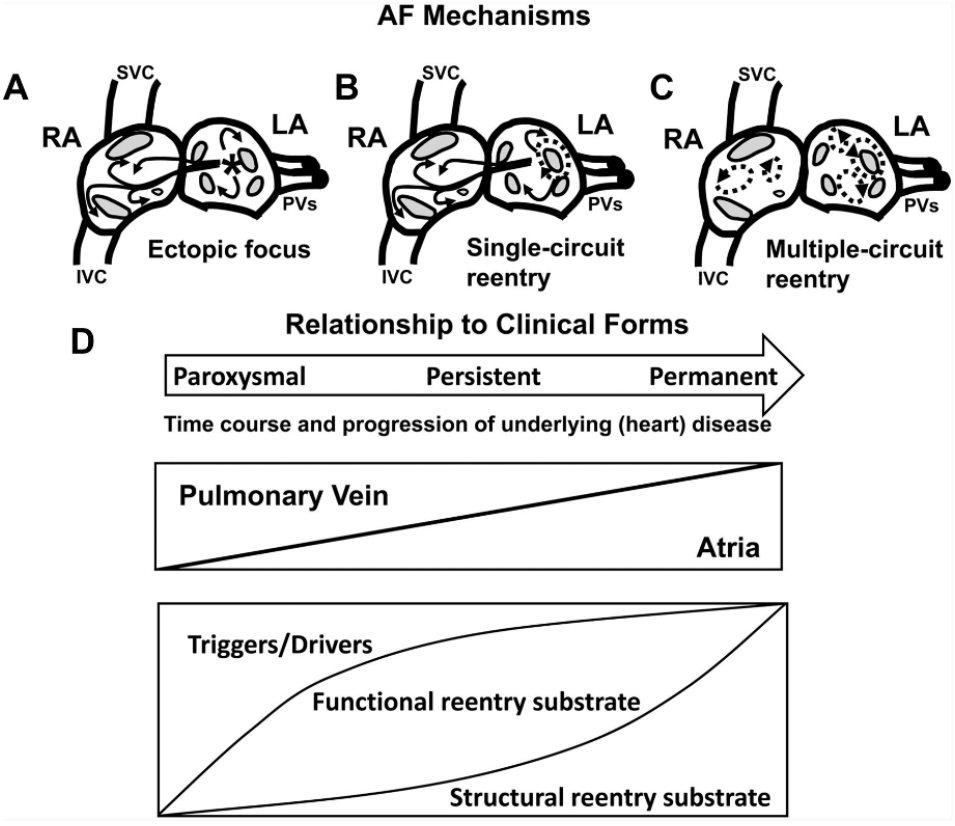
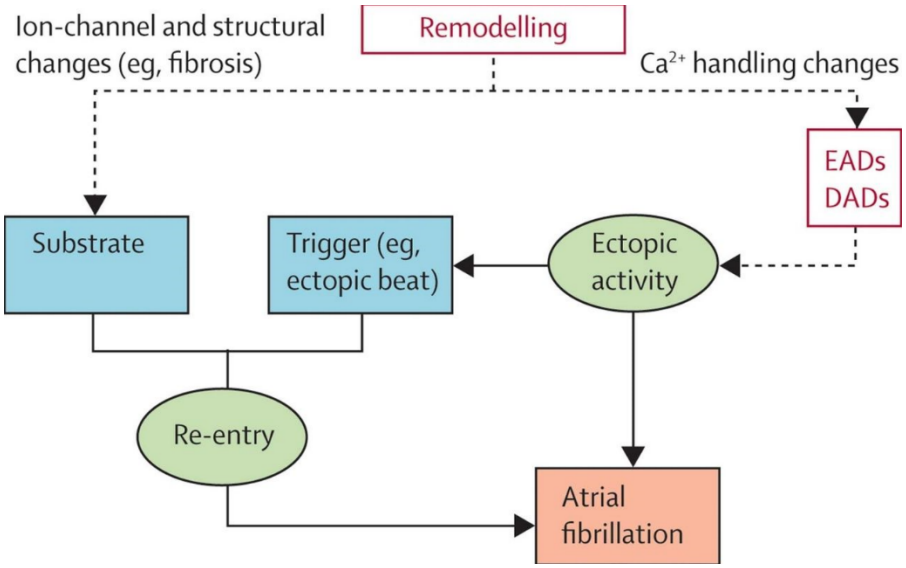


Figure 2-2. Key concepts underlying the induction and maintenance of atrial fibrillation (AF). AF can be maintained by either re-entrant or rapid and sustained ectopic activity. Development of re-entry depends on the action of a trigger (usually from an ectopic beat) acting on vulnerable substrate. In normal hearts, atrial electrical properties are less likely to support the maintenance of AF. Atrial remodelling creates a substrate for re-entrant AF, by altering ion channel function and/or inducing tissue fibrosis. Remodelling can also make ectopic activity more likely by producing changes in Ca^{2+} handling that promote both triggered activity and re-entry. DAD, delayed after depolarisation; EAD, early after depolarisation (32).



2.1.3 Morbidity and mortality

It is estimated that 25-30% of AF cases are asymptomatic or silent (33) and between 25-62% are paroxysmal (34,35). Consequently, AF frequently goes undetected and untreated and in up to 30% of patients is only detected at the time of thromboembolic complications, such as stroke (24,33,36,37). Episodes of AF can result in electrophysiological and structural changes to the atria that increase the chances of more frequent episodes or sustained AF (24,38). As a result, AF can

progressively worsen overtime, increasing the risk of morbidity and mortality and imposing a significant burden on patients and substantial costs to the health care system (27).

Typically, AF-related mortality is due to resulting comorbidities and complications, rather than the arrhythmia itself (39,40). In a large meta-analysis including 104 cohort studies, AF was significantly associated with an increased risk of all-cause mortality (pooled relative risk [RR] 1.46; 95% CI 1.39 – 1.54), cardiovascular mortality (pooled RR 2.03; 95% CI 1.79 – 2.30), major cardiovascular events (pooled RR 1.96; 95% CI 1.53 – 2.51), ischaemic heart disease (pooled RR 1.61; 95% CI 1.38 – 1.87), sudden cardiac death (pooled RR 1.88; 95% CI 1.36 – 2.60), heart failure (pooled RR 4.99; 95% CI 3.04 – 8.22), chronic kidney disease (pooled RR 1.64; 95% CI 1.41 – 1.91), and peripheral arterial disease (pooled RR 1.31; 95% CI 1.19 – 1.45) (41). AF also increases the risk of stroke 5-fold (1) and is the leading cardiac cause of stroke, which is the second leading cause of death worldwide (42). Moreover, AF-related stroke is associated with higher mortality, longer hospitalizations, greater incidence of stroke recurrence within a year, and greater neurological and functional disability compared to non-AF related stroke patients (24,33,43–46). As stroke increases the risk of dementia 2-fold (5), stroke can act as an intermediate between AF and dementia. **However, there is strong evidence for associations between AF and dementia, independent of stroke, highlighting a direct relationship between AF and cognition, which is outlined in more detail in Section 2.3. below** (6,47).

2.2 Cognitive Decline and Dementia

2.2.1 Definition and burden

Worldwide, more the 55 million people have dementia and dementia is a leading cause of disability and dependency among older adults, with devastating physical, psychological, social, and economic impacts on patients, caregivers, and health systems (4). In 2019, the total global societal

cost of dementia was US$1.3 trillion, 50% of which is attributed to informal care (4). According to the National Population Health Study of Neurological Conditions, the total Canadian health care system and out-of-pocket caregiver costs amounted to $10.4 billion in 2016 (48). Dementia is a chronic and progressive syndrome in which multiple-domain cognitive impairment, including memory and other domain-specific impairment, is sufficiently severe to significantly affect everyday functional abilities (49).

2.2.2 Mixed dementia: Vascular contributions to dementia

The most common diagnosis of dementia is Alzheimer's Disease (AD) characterized by progressive memory decline and impairment of executive and visuospatial function (26, 27). Vascular dementia (VaD) is considered the second most common cause and is diagnosed when vascular events disrupting cerebral blood flow are responsible for cognitive decline (49). It often presents as an acute onset and stepwise decline, where executive dysfunction predominates (49). While previously estimated to contribute to 15-20% of cases (50), emerging data suggest that VaD is grossly underestimated, as vascular events and pathology often go undetected (49). Moreover, mixed pathologies, in which patients show signs of both AD and VaD is increasingly recognized as the most common etiology of dementia in older adults (51–53). In a longitudinal study including 483 autopsied participants, more than half of those with AD had a combination of both AD and vascular pathologies (51,54).

Typically, dementia has a long pre-symptomatic phase, providing an optimal window to aggressively manage vascular risks, slow progression, and enable longer independent living (55). However, dementia often goes undiagnosed due to its insidious onset (56), highlighting the need to identify biomarkers for early detection (55). Despite numerous treatment trials and research efforts, there is not a single disease-modifying drug for dementia (21). Many of these unsuccessful

drug candidates aimed to reduce Aβ plaques in the brain via amyloid-lowering therapies based on the "amyloid hypothesis", the leading hypothesis of AD pathogenesis for 25 years (21). While this hypothesis was based on the assumption that an increase in the ratio of cortical Aβ-42 induces Aβ fibril formation, leading to senile plaque development, neurotoxicity and ultimately neural cell death and neurodegeneration; no clinical trials ever established efficacy of amyloid-lowering therapies and recent evidence from animal models and amyloid imaging have confirmed that Aβ deposition has no relationship to the onset of AD (57). Further, available therapeutics, such as acetylcholinesterase inhibitors, that aim to reduce dementia-related symptoms, have had modest effects at best (58,59). In the absence of treatment options, there is a critical need to discover new therapeutic targets. Critically, AF was independently associated with both AD and VaD in the prospective Intermountain Heart Collaborative study including 37,025 patients (60). Given the considerable overlap between AD and VaD and the potential role of vascular dysfunction, vascular conditions such as AF, may be important contributors to incident dementia.

2.3 Associations Between Atrial Fibrillation and Dementia

Many population-based studies have shown associations between AF and dementia or cognitive decline. Among 6,514 dementia-free participants in the prospective Rotterdam Study, prevalent AF was related to a 30% increase in the hazard of dementia, independent of stroke (hazard ratio [HR]: 1.33; 95% CI: 1.02 – 1.73) (61). Further, longitudinal prospective studies with repeated measures of cognitive function also demonstrate an association between AF and an increased risk of cognitive decline (62–64). In a recent meta-analysis including 21 studies, AF was significantly associated with a higher risk of cognitive impairment and dementia in stroke patients (RR: 2.70; 95% CI: 1.82 – 4.00) and in the broader population with or without stroke (RR: 1.40; 95% CI: 1.19 – 1.64) (6). Clinical prediction tools have been developed to estimate the risk of stroke in patients

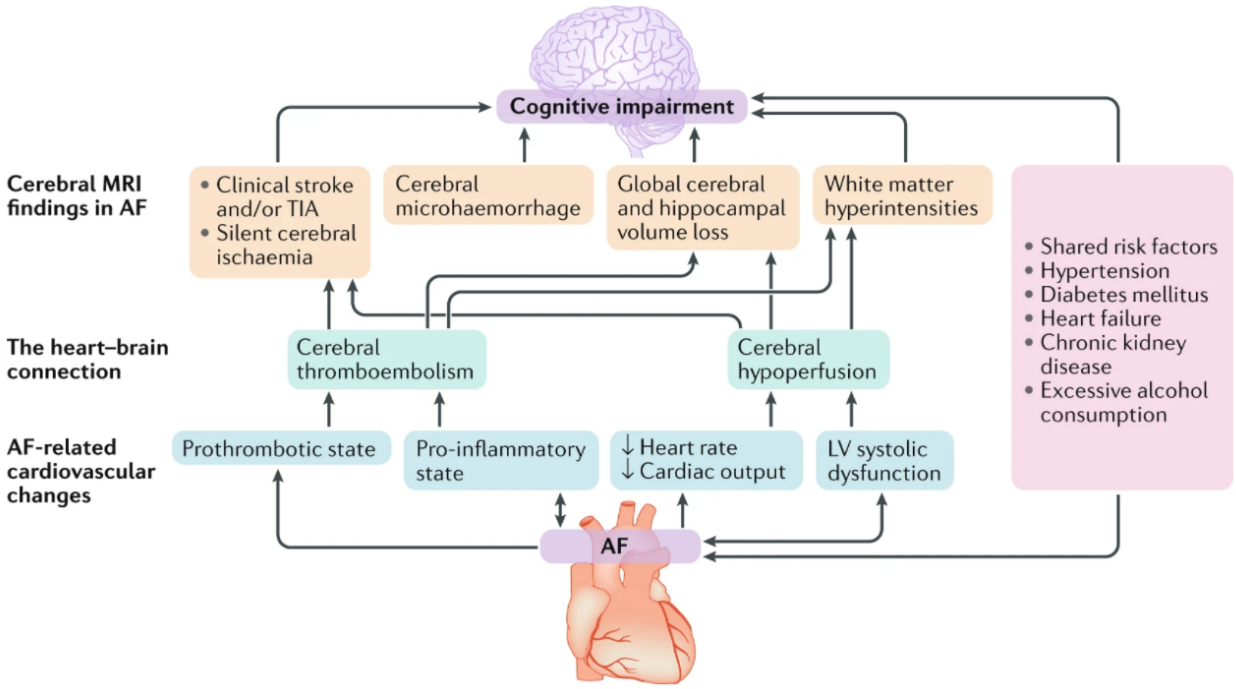
with AF and inform decision-making for anticoagulant treatment. Notably, the most commonly used tool for stroke risk is the congestive heart failure, hypertension, age > 75 years, diabetes, stroke ($CHADS_2$) score and the updated CHA_2DS_2-VASc score, which incorporates female sex, vascular disease, and age 65-74 as additional risk factors (65). In a large-scale population-based cohort study among 332,665 AF subjects, these scores were also significant predictors of dementia with an adjusted hazard ratio of 1.52 and 1.5 per 1-point increment of the $CHADS_2$ and CHA_2DS_2-VASc scores, respectively (66). Furthermore, treatment of AF with oral anticoagulants may preserve cognitive function. In a registry-based study of nearly half a million patients with AF, oral anticoagulant treatment was associated with a 29% decrease in dementia risk compared to those without treatment (22).

Interestingly, among these studies, some have found that AF significantly increased the risk of dementia among younger subjects below 75 years of age but not in older subjects (60,61,67). Analyses of the Rotterdam Study demonstrated that younger participants with the longest history of AF had the highest risk of dementia, suggestive of a dose-response relationship (61). Therefore, as dementia progresses over many years, exposure to AF may have to occur at a younger age to lead to dementia onset (7). Another plausible explanation is that since cognitive function progressively deteriorates over time, those in older age groups with dementia are often censored, biasing the results towards the null. This may also explain why other longitudinal studies including individuals over the age of 75 years found no association between AF and dementia (68–70). In addition, these studies may not have been powered to detect an association due to smaller sample sizes and shorter follow-up periods in older age strata. Despite strong evidence for these associations, the potential pathways linking AF and dementia remain unclear.

2.4 Proposed Pathophysiological Pathways Linking Atrial Fibrillation and Dementia

Several pathways have been proposed as contributing to the association between AF and dementia (see Figure 2-3 below). Four primary pathways highlighted by *Madhavan et al. (2018)* will be reviewed: shared risk factors, prothrombotic state and cerebral microbleeds, cerebral hypoperfusion, and pro-inflammatory state.

Figure 2-3. Proposed pathophysiology of cognitive impairment in atrial fibrillation. Atrial fibrillation (AF) has a number of effects that predispose individuals to cognitive impairment, including a prothrombotic state, a pro-inflammatory state, and reduced cardiac output. Final pathways leading to cognitive impairment potentially include cerebral embolism, haemorrhage, and volume loss. LV, left ventricular; TIA, transient ischaemic attack (7).



2.4.1 Pathway 1: Shared risk factors

First, a noncausal explanation draws on the common etiology shared by both AF and dementia (7). AF and dementia share multiple risk factors, particularly advancing age (7). Other shared risk factors include excessive alcohol consumption (71,72) and comorbidities, such as hypertension, diabetes, heart failure, and chronic kidney disease (7,25,73–77). However, as previously highlighted, prospective studies have demonstrated a significant temporal relationship between AF and dementia after adjustment for these shared risk factors (7). Although residual confounding is possible, it is unlikely to account for the consistent results across the literature. In a meta-analysis, *Kalantarian et al. (2013)* examined the association between AF and cognitive impairment independent of stroke and showed that a sensitivity analysis excluding studies with inadequate adjustment for potential confounders had little effect on the reported results (6). Further, a dose-response relationship between AF and dementia in younger subjects has previously been established (78), supporting a causal association and highlighting that other potential pathways contribute to this relationship.

2.4.2 Pathway 2: Prothrombotic state (or silent cerebral infarcts) and cerebral microbleeds

Second, AF also produces a prothrombotic state. It is thought that the components of Virchow's triad are responsible for increasing the risk of thromboembolism: blood stasis, hypercoagulable state, and endothelial dysfunction (14). First, the contractile dysfunction characteristic of AF reduces cardiac output and leads to blood stasis (79). Second, there is an increased activation of the coagulation cascade, platelet reactivity, and impaired fibrinolysis (14). Third, AF induces structural changes including atrial fibrosis and endothelial dysfunction (14). Together, these three changes promote thrombus formation and subsequent embolization (14). Although studies have shown an association between AF and dementia, independent of clinical cerebrovascular events

(e.g., stroke), silent cerebral infarction (SCI) (i.e., asymptomatic stroke) may also act as an intermediate variable for this association (7,80) and has been associated with both cognitive decline and dementia. In the prospective Rotterdam Scan Study, the presence of SCI at baseline more than doubled the hazard of dementia (HR 2.26; 95% CI 1.09-4.70) and was associated with greater cognitive decline compared to those without infarcts (81). AF has also been associated with SCI on MRI (82), and those with both AF and SCI had higher odds of mild cognitive impairment when compared to those without (83). However, other studies demonstrated that AF was associated with lower brain volume and greater cognitive decline independent of SCI on brain imaging techniques (84,85). Of note, these studies were all cross-sectional, so future prospective studies are required to establish SCI as a true mediator.

In patients with AF, thromboembolic risk is typically managed with the use of oral anticoagulant therapy (24). Although this intervention significantly reduces the risk of embolic stroke, it increases the risk of intracerebral haemorrhage and cerebral microbleeds (86). In the Rotterdam Study, a high microbleed count significantly increased the risk of cognitive decline and dementia (87). However, given that the annual rate of treatment-related bleeding events is estimated to be 0.1-2.5% and that anticoagulation treatment has been shown to reduce the risk of dementia (22), cerebral microbleeds are unlikely to independently account for this relationship (7).

2.4.3 Pathway 3: Cerebral hypoperfusion

Cerebral hypoperfusion and hypoxia has also been proposed as a pathway underlying the association between AF and dementia (7,13), as the brain has a high metabolic demand and requires a steady supply of blood (88). In patients with AF, reduced cardiac output and left ventricular dysfunction due to irregular cardiac rhythm can result in cerebral hypoperfusion and hypoxia (7,89,90). In the AGES-Reykjavik Study, those with persistent AF had decreased cerebral

blood flow and brain perfusion when compared to those without AF (91). Further, a linear relationship was found between increasing duration of AF and lower total brain volume (84), although other studies found conflicting results (92,93). Cerebral hypoperfusion has also been associated with dementia and subcortical white matter hyperintensities on MRI (7,94). In the Framingham Heart Study, lower left ventricular ejection fraction was associated with lower mean cognitive performance (95). In individuals with AD, decreased cerebral blood flow has been observed prior to Aβ deposition in the brain, a peptide which, when accumulated in the extracellular space, is a hallmark of AD pathology (51). AF is thought to impair the clearance of Aβ and contribute to the progression of cognitive decline (51).

Logically, interventions that restore sinus rhythm in individuals with AF should then improve cerebral perfusion and thus cognitive outcomes, however results have been mixed, suggesting that this association may not be purely driven by thromboembolic or hypoperfusion pathways (7). In the large prospective Intermountain AF study, those with AF who underwent catheter ablation had a significantly lower risk of dementia compared to those with AF who had not received ablation (96). Contrary to that study, randomized trials have shown that rhythm control therapies, including cardioversion, anti-arrhythmic drugs, and ablation, do not improve cognitive function, as measured by the Montreal Cognitive Assessment and Mini-Mental State Examination (97,98).

2.4.4 Pathway 4: Pro-inflammatory state

Another proposed pathway contributing to this association is the pro-inflammatory state detected in both AF and dementia (89). Several studies have highlighted the association of systemic inflammatory markers with the initiation and perpetuation of AF (8). IL-6 is a cytokine in the plasma that mediates the inflammatory response and induces the hepatic synthesis of CRP and

fibrinogen, acute-phase inflammatory proteins (99). Turbulent and static flow in the atrium resulting from AF can cause endothelial damage, increasing the release of inflammatory markers (90,100,101), including IL-6, CRP, and fibrinogen (14–18). Growing evidence supports inflammation as an important contributor to neurovascular and neurodegenerative changes and cognitive decline (9–11). Post-mortem brain tissue studies of individuals with AD found chronic inflammation, as indicated by activated neuronal immune cells, called microglia, around Aβ plaques and neurofibrillary tangles (9,51). Further, another study suggested that the plaques and tangles characteristic of AD only led to neurodegeneration when inflammation was present (51,102). Therefore, systemic inflammation is a promising pathway linking AF with cognitive decline and dementia and will be the focus of this thesis.

2.4.5 The missing link: Blood-brain barrier dysfunction

The pathway by which systemic inflammation in AF directly impacts the brain remains to be elucidated. A potential mediator of this relationship and a large evidence gap in the model depicted in Figure 2-3 is the potential role of the BBB.

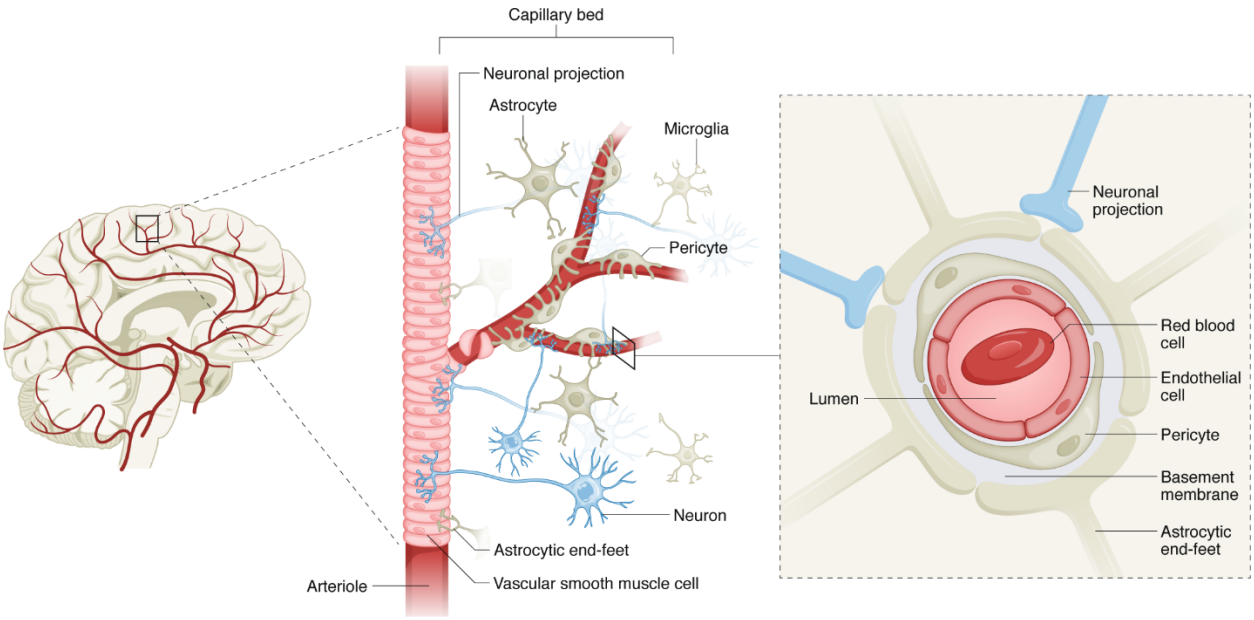
Blood-brain barrier structure and function

The BBB is a highly regulated structure separating the brain parenchyma from the systemic circulation (13). A functional BBB maintains brain homeostasis by preventing the entry of most blood-derived molecules, including pathogens, neurotoxins, and xenobiotics (13). Therefore, maintaining its integrity is critical to ensure proper neuronal function and to protect the CNS from inflammation, injury, and disease (103).

Unlike non-neural tissues, the blood vessels vascularizing the CNS are composed of non-fenestrated endothelial cells held together by tight junctions and possess a low number of pinocytic vesicles and leukocyte adhesion molecules that limit transcytosis and the entry of immune cells,

maintaining strict control over the passage of molecules, ions, and cells between the circulation and the brain (13,104,105). Endothelial cells have unique transport properties, including efflux transporters that remove lipophilic toxins able to passively diffuse across the cell membrane and nutrient transporters that allow the entry of important nutrients into the brain such as water, glucose, and amino acids, as well as remove waste products (13,106). The BBB is a multicellular system supported by pericytes, astrocytes, a basement membrane, microglia, and neurons, together forming the neurovascular unit (see Figure 2-4 below). The endothelium is partially covered by a layer of pericytes, cells responsible for regulating angiogenesis, immune cell infiltration, blood flow, and BBB formation and function (107). Astrocytes are a major glial cell in the CNS that extend processes to link neurons with surrounding blood vessels, allowing them to regulate cerebral blood flow and vasomotor responses (108,109). In addition, they have been shown to regulate BBB junctions and transport (104). The basement membrane, surrounding the endothelium and pericytes, functions as a second barrier and supports interactions between the endothelial cells, pericytes and astrocytes (109). Microglia are the sole immune cells of the CNS that closely monitor the brain parenchyma to maintain homeostasis, remove debris, and support neuronal development and repair (110). As a healthy CNS contains very few immune cells and tightly controls their passage, it is typically an immunosuppressed environment (108).

Figure 2-4. Structure of the BBB. At the level of the penetrating arteries, endothelial cells are surrounded by vascular smooth muscle cells that regulate vascular tone. At the level of the intracerebral capillaries, the neurovascular unit is comprised of endothelial cells, pericytes, astrocytes, basement membrane, microglia, and neurons. Continuous non-fenestrated endothelial cells are held together by tight junctions and pericytes partially encase the endothelium, which are both surrounded by a common basement membrane. Astrocyte endfeet completely surround the capillary wall and link to nearby neurons. Microglia are in constant surveillance in the brain parenchyma (109,111).



BBB Dysfunction

Evidence of BBB dysfunction has been demonstrated in multiple neurodegenerative disorders, including multiple sclerosis, Huntington's disease, Parkinson's disease, and dementia (105,106,112–114). Cardioembolic stroke (i.e., AF-related stroke) has also been associated with BBB permeability (115). Critically, a recent study showed that AF was associated with higher

levels of Tau protein and glial acidic fibrillary protein in the systemic circulation, biomarkers of neuronal injury and disruption of the BBB (116). BBB dysfunction may result from disruption of tight junctions, accumulation of blood-derived products, degeneration of pericytes and endothelial cells, increased transcytosis, disrupted transport, or increased leukocyte infiltration (104,105). As a result, the breakdown of the BBB impairs the transport of nutrients to the brain and allows the entry of neurotoxic blood-derived molecules into the CNS, activating central inflammatory and immune responses that further perpetuate the impairment of the BBB in a vicious cycle (12,106).

Increased inflammation can lead to BBB dysfunction by downregulating tight junctions, altering transcytosis and transporters, degrading the basement membrane and disrupting astrocyte endfeet (108). Therefore, systemic inflammation in AF may lead to the breakdown of the BBB, predisposing patients to cognitive impairment and dementia (9–11,106). Further, an increased demand for oxygen due to cerebral hypoperfusion can result in the production of reactive oxygen species (ROS) (13,117). The upregulation of ROS stimulates cells of the BBB to release inflammatory factors that can further impair the BBB (117). When cytokines, such as IL-6, infiltrate the brain, they cause demyelination and damage to neuronal cells associated with the hippocampus and white matter (12). In addition, fibrinogen is highly neurotoxic and, while typically blocked by the BBB (90,118), if the BBB is damaged it can enter into the CNS, co-localize with Aβ, and lead to axonal retraction, and promote microglia activation and neuroinflammation (106,119). Increased blood levels of this protein have been associated with a higher risk of AF, dementia, and cognitive decline (18,120,121). Further, plasma IL-6, CRP, and fibrinogen have been associated with BBB dysfunction (19,20,118,122–124). **Therefore, leakage of these systemic inflammatory markers through the BBB represents a potential pathogenic**

pathway of neurovascular and neurodegenerative changes in individuals with AF, but few studies have examined its role in the association between AF and cognitive decline.

2.5 Conceptual Framework

Figure 2-5 below depicts our conceptual framework of the hypothesized pathways linking AF to cognitive decline and dementia. Based on previous literature, we have identified a set of variables and the proposed relationships between them.

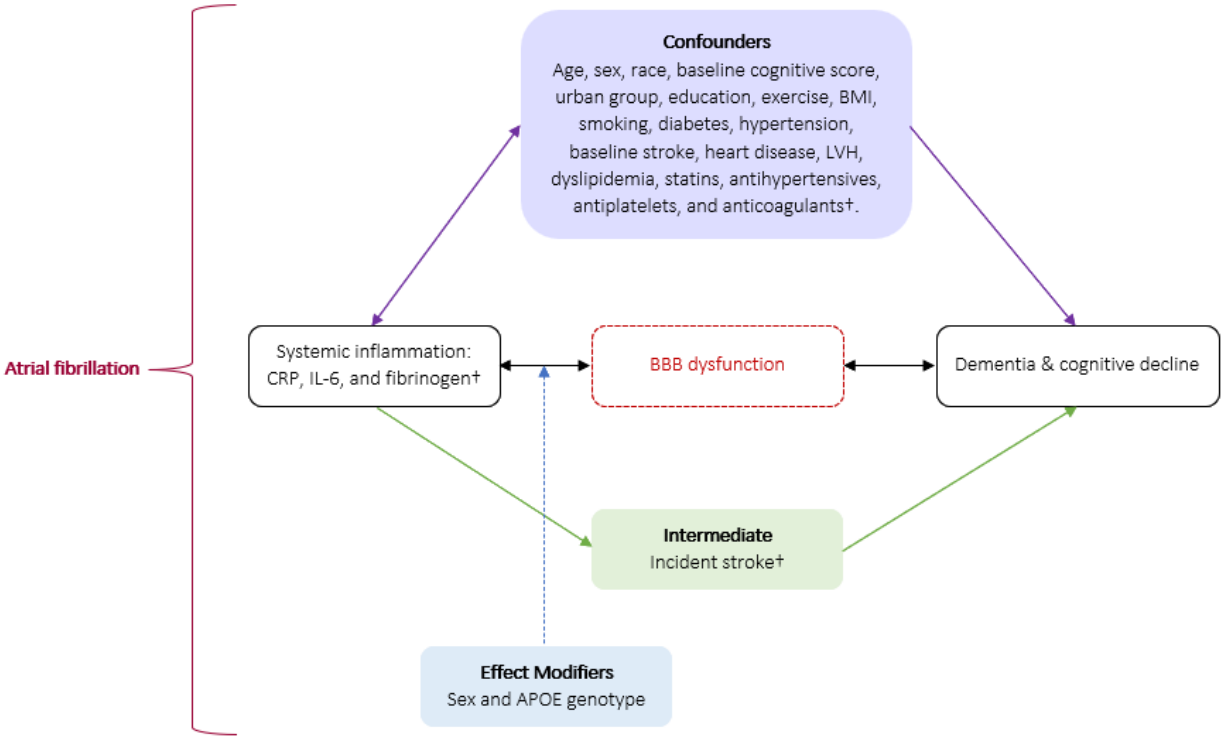


Figure 2-5. Conceptual framework of the proposed contributing pathways linking AF to cognitive decline and dementia. Systemic inflammation contributes to the initiation and perpetuation of AF which in turn increases systemic inflammation. CRP, IL-6 and fibrinogen, markers of systemic inflammation associated with BBB dysfunction, will be used as the exposure variables in models. The resulting inflammatory-mediated BBB dysfunction then leads to cognitive decline and dementia our outcome variables of interest. Confounders of the exposure-

outcome relationship have been listed based on background knowledge and will be included as covariates in adjusted models. As indicated by unidirectional arrows, incident stroke is hypothesized as an intermediate of the exposure-outcome relationship. We will also investigate sex and APOE genotype as potential effect modifiers to establish the need to report sex-specific estimates or model APOE interaction terms. BMI, body mass index; LVH, left ventricular hypertrophy; CRP, C-reactive protein; IL-6, Interleukin-6; BBB, blood-brain barrier; APOE, apolipoprotein E. †Entered as a time-varying variable in survival models where possible.

Chapter 3. Additional Methods

As some pertinent details relating to the study methodology have been excluded from the manuscripts to better align with requirements for journal submission, this chapter will provide relevant supplemental methodological information.

3.1 Data Sources

Analyses in the primary thesis manuscripts (Chapter 4 and 5) used data from the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study and the Cardiovascular Health Study (CHS) cohorts (see *Appendix A* for depiction of study designs).

3.1.1 Reasons for Geographic and Racial Differences in Stroke (REGARDS) Study

The REGARDS study was a prospective population-based cohort study of 30,183 adults aged ≥ 45 years in the United States (125). The sample was selected from commercially available nationwide lists of residents using mail and telephone contact. Fifty-five percent were recruited from the Stroke Belt, a region of high stroke mortality including 8 southeastern states: North Carolina, South Carolina, Georgia, Tennessee, Mississippi, Alabama, Louisiana, and Arkansas. The others were recruited from the remaining states (125). Participants were enrolled between

January 2003 and October 2007 (126). Inclusion criteria included having a name, telephone number, and address in the listings (125). Potential participants were excluded if they were a race other than Black or White, were undergoing active treatment for cancer, had medical conditions that would prevent long-term participation, were cognitively impaired, resided in or were on a waiting list for a long-term care home, or were unable to communicate in English (125). Eligible participants providing verbal consent underwent a baseline computer-assisted telephone interview to collect data on demographics, risk factors, and medical history (125). At a subsequent in-home visit, a trained health professional obtained written informed consent, performed physical measurements, a medication inventory, a resting electrocardiogram (ECG), and a blood and urine collection (125). Participants or their proxies were contacted by telephone at 6-month intervals with retrieval of medical records over a maximum follow-up period of 17 years (125).

Primary outcome ascertainment: Dementia

The primary outcome was incident dementia, ascertained by trained technicians by telephone at baseline and annually over follow-up using the Six-Item Screener (SIS). The SIS assesses global cognitive function and includes a three-item recall and a three-item temporal orientation (day of the week, month, year) (127) with a score ranging from 0 to 6. Dementia was defined as a score of 3 or less at any follow-up assessment, a cut-off previously shown to have a sensitivity of 88.7% and specificity of 88.0% for the diagnosis of dementia (127). It is important to note that this is a screener that, while shown to be valid for dementia diagnoses, does not involve all measures included in the clinical criteria for dementia, such as comprehensive neuropsychological testing or assessment of activities of daily living.

3.1.2 The Cardiovascular Health Study (CHS)

The CHS was a prospective cohort study which was conducted to determine risk factors and consequences of cardiovascular diseases in older adults in four communities in the United States: Sacramento County, California; Washington County, Maryland; Forsyth County, North Carolina; and Pittsburgh, Pennsylvania (128). CHS enrolled 5,888 adults aged 65 or older (an original cohort of 5,201 in 1989-90 and a supplemental cohort of 687 predominantly Black participants in 1992-93) (128). Eligible participants were sampled from Medicare eligibility lists in each area (128). Participants were excluded if they could not give consent or answer questions without a surrogate, resided in an institutional setting, were wheelchair-bound, were receiving hospice treatment, or were receiving active treatment for cancer (128). Participants underwent extensive physical and laboratory baseline examinations and were followed through 1998-99 with annual clinical examinations, telephone interviews at 6-month intervals, and data were linked with Medicare claims (128).

In 1998-99, an ancillary study, the Cardiovascular Health Cognition Study (CHCS), was initiated to classify dementia status among 3,608 CHS participants who had a brain MRI in 1991-94 and a cognitive test in the same year (129). A second brain MRI was taken in 1997-98 (130). Participants who received an MRI were significantly younger, had a higher education and income, were less likely to smoke and to have had prior cardiovascular disease, hypertension, or diabetes mellitus compared to those who did not receive an MRI scan (129).

Primary outcome ascertainment: Dementia

The primary outcome was incident dementia. Detailed information regarding dementia classification has been previously described (129,130). In brief, using the data collected between 1989 and 1999, the participants from the CHCS were classified as high or low risk for dementia

(130). High risk was defined as one of the following: (1) a Modified Mini-Mental State Examination (3MS) score < 80, (2) ≥ 5-point decline on the 3MS from the time of MRI, (3) a Telephone Interview for Cognitive Status (TICS) score < 28, (4) an Informant Questionnaire for Cognitive Decline in the Elderly (IQ CODE) score > 3.6, (5) incident stroke, (6) a medical record with dementia diagnosis, or (6) residing in a nursing home (130).

At 3 of the sites, surviving high-risk White participants and all surviving Black participants were brought into the clinic for neuropsychological testing (130). At the Pittsburgh site, all surviving participants were evaluated regardless of risk status or race (130). In clinic, participants underwent an extensive neuropsychological battery (130). Those who failed tests of memory or more than one cognitive domain were further examined by a neurologist (129).

A committee of neurologists and psychiatrists classified all participants based on all the available data (129). Participants who did not meet dementia criteria but who presented cognitive deficits were classified with mild cognitive impairment (MCI) (129). For participants unable to come into the clinic or who were deceased, dementia was assessed using existing CHS data collected from the annual clinical examinations, medical records, physician questionnaires, and proxy interviews (129). Six participants were not classified due to a lack of sufficient information (129). Dementia onset was determined by review of the data collected over the course of follow-up and by family input using the Neuropsychiatric Inventory (129). If the date of onset was before entry into the CHCS, the participant was classified as prevalent dementia at baseline (129).

3.2 Study Design and Cohort Selection

To determine whether inflammatory-mediated BBB dysfunction causally contributes to cognitive decline and dementia in adults with AF, our goal was to investigate the association of markers of

BBB dysfunction, including IL-6, CRP, and fibrinogen, with cognitive decline and incident dementia in a cohort of aging adults with prevalent AF at baseline.

To establish a temporal sequence between AF and cognitive outcomes, we used a prospective cohort design and excluded those with cognitive impairment or dementia at baseline. The specific exclusion and inclusion criteria are detailed in the manuscripts. Our sample size calculations for both cohorts are detailed in *Appendix B*. A minimum sample size of 2,529 individuals in the REGARDS cohort was required to be sufficiently powered to detect a 20% increase in hazard. In the REGARDS cohort, we were slightly underpowered (power = 69.6%) as 2,109 participants had AF at baseline and no cognitive impairment. In the CHS cohort, a minimum sample size of 2,570 individuals was required. However, there were only 70 prevalent AF cases and 1,104 incident AF cases (505 of which occurred prior to dementia diagnosis). To mitigate the impact of reduced power due to this low sample size of prevalent AF cases, we expanded the cohort to include all individuals, and stratified based on AF status over the study follow-up (N=3,375). Estimates of association were obtained for the overall cohort and stratified by AF status.

3.3 Descriptive Analyses

Descriptive analyses of the variables of interest were conducted prior to proceeding with the primary analyses. For categorical variables, distributions were summarized using frequency tables. For continuous variables, histograms, box plots, and measures of central tendency and variability were used to summarize distributions. No outliers or coding errors were identified in the variables of interest. The frequency and percentage of missing observations were summarized for each variable. In the REGARDS cohort, IL-6 was included in our pre-specified analytic plan as a secondary exposure variable, however, 91.6% of the observations were missing in the analytic

cohort. We therefore restricted our analyses to CRP as the only exposure. Other variables with extensive missingness in the REGARDS cohort included APOE Ɛ4 (91.3%) and medication use at follow-up (59.4%) and were thus excluded from these analyses.

For continuous variables, skewness of the distribution was evaluated by visual inspection of histograms and interpretation of skewness and kurtosis values. In the REGARDS cohort, CRP displayed a right-skewed distribution (Skewness: 6.7; Kurtosis: 74.1). In the CHS cohort, CRP and IL-6 displayed right-skewed distributions (Skewness: 9.50 and 7.3; Kurtosis: 137.1 and 127.1, respectively). To correct for skewness, these variables were $\log_2$ transformed. Fibrinogen was scaled down by a factor of 100 to enable interpretation of effect estimates. Chi-square tests, t-tests, Pearson's correlations, Analysis of Variance (ANOVA), and Wilcoxon-rank sum tests were used to examine potential collinearity between the covariates and associations with the exposure and outcome variables.

3.4 Model Selection

3.4.1 Competing risks cause-specific Cox proportional hazards model

Our analytic goal was to estimate the risk of incident dementia as a function of time in individuals with AF at differing levels of circulating inflammatory markers. We therefore selected a Cox proportional hazards regression model to analyze incident dementia as a time-to-event outcome. As the occurrence of death precluded the occurrence of dementia in both cohorts (REGARDS n = 861 and CHS n = 2,479), we selected a competing risks time-to-event framework. As outlined by Lau et al (131), the choice between the Fine-Gray subdistribution hazard model and the cause-specific hazard model should be guided by the study objective. Our objective was to identify whether inflammatory markers were associated with an increased hazard of the occurrence of dementia in subjects who are currently event-free. We therefore selected a cause-specific hazard

model as it is better suited for studying etiologic questions, whereas the subdistribution hazard model is better suited for predicting prognosis and estimating the incidence of the outcome (132). This model lends itself well to etiologic interpretations as, in the presence of competing risks, we can interpret the magnitude of the effect of inflammatory markers on the rate of occurrence of dementia from the cause-specific hazard ratio, but not from the subdistribution hazard ratio (132). Furthermore, in the presence of competing risks, the Kaplan-Meier estimates of survival function results in an upward bias of the event incidence (132). Therefore, we plotted cumulative-incidence function curves to estimate the crude incidence of dementia and death by exposure category, as this method accounts for competing risks (132).

3.4.2 Longitudinal model: Latent class growth analysis and logistic regression

Additionally, within both cohorts, we were interested in modeling the longitudinal cognitive trajectories of individuals over time and aimed to investigate whether distinct growth trajectories existed within the overall cohort. We used latent class growth analysis (LCGA) to identify subgroups of individuals based on their common cognitive trajectories over time. We used the following analytic approach outlined by Wardenaar (2020) (133):

1. Define the underlying growth model: cognitive score as a function of time (days).
2. Models of increasing number of classes (1 to 3 classes) were fit to the data.
3. Compute and plot the predicted values of the longitudinal cognitive score in each latent class.

In both the REGARDS and the CHS cohorts, the optimal models included two latent classes, one class with a stable cognitive trajectory (slope = 0.00001 and 0.00015, respectively) and the other displaying a trajectory of cognitive decline (slope = -0.00091 and -0.0061, respectively). We selected binomial logistic regression to model the relationship between inflammatory markers and

latent class membership, adjusting for confounders. Further details are provided in the manuscripts (Chapters 4 and 5).

3.5 Checking Model Fit and Assumptions

3.5.1 Competing risks cause-specific Cox proportional hazards model

A key assumption of the Cox model is the proportionality of hazards, in which the relative hazard for any two subjects remains constant over time (134). To test this assumption, we used Schoenfeld residuals tests to examine the statistical significance ($p<0.05$) of each covariate and for the global models. A significant test statistic indicating non-proportionality was further visually inspected using plots of the scaled Schoenfeld residuals versus time to check for any patterns or departures from the horizontal line. As indicated by the global test statistics, the models did not violate the proportional hazards assumption. In the REGARDS cohort, baseline cognitive score was the only covariate that violated the proportional hazards assumption (see *Appendix C* for Schoenfeld plot). To resolve this violation, we entered baseline cognitive score into the model using the strata function which relaxes the proportional hazards assumption and allows separate baseline hazard functions to be estimated within each strata. There were no covariate violations in the CHS models.

Another assumption of the model is that the relationship between the log-hazard and each continuous covariate is linear (134). To test this assumption, martingale residuals were plotted against continuous covariates to detect non-linearity (see *Appendix D* for plots of martingale residuals versus age). Finally, to check for influential observations or outliers, we inspected plots of *dfbeta* and deviance residuals. Residuals were roughly symmetrically distributed about the 0 and none of the observations were individually influential.

3.5.2 Longitudinal model: Latent class growth analysis and logistic regression

To identify the optimal latent class growth models, we compared class sizes and indices of fit between models of increasing number of latent classes. We used the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) fit indices, with the lowest values indicating the best model. In addition, for the CHS study, we re-ran latent class analyses within sex strata to investigate differences in classification. Compared to classification in the overall cohort, 37 females were reclassified as stable, and 20 males were reclassified as cognitive decline.

A key assumption of the logistic regression model is that there is a linear relationship between the logit of the outcome and each continuous predictor or covariate. To verify that this assumption was met, we visually inspected scatter plots of each continuous variable versus the logit of the outcome. We checked for influential observations and outliers by visually inspecting standardized residuals and Cook's distance values. Finally, we did another inspection of multicollinearity among variables by computing variance inflation factors.

3.6 Handling Missing Data

We followed the Treatment And Reporting of Missing data in Observational Studies (TARMOS) framework to select the appropriate method for handling missing data among both cohorts (135). To further explore the patterns of missingness in both cohorts, we created two dummy variables: the first, indicating whether the exposure variable was missing and the second, indicating whether any covariate value within a record was missing. We then conducted t-tests, Chi-square tests, and Wilcoxon Rank-Sum tests to verify whether the exposure, outcome, and covariate variables were significantly associated ($p<0.05$) with the indicators of missingness. In the REGARDS cohort, missingness in CRP was significantly associated with dyslipidemia and diabetes, however these variables also relied on blood assays. In CHS, there was a small percentage of missing exposure

and covariate values (<10% of records) and missingness was not associated with dementia in exploratory analyses. We therefore conducted complete case analyses, as it is presumed to provide an unbiased estimate of the exposure-outcome relationship if the reasons for missingness in any variable is unrelated to the outcome (135). Furthermore, multiple imputation would likely not increase efficiency given the small percentage of missingness in the CHS cohort and in the absence of strong auxiliary variables for CRP in the REGARDS cohort.

For longitudinal analyses of cognitive decline in the CHS cohort, there were intermittent missing cognitive scores at annual follow-up assessments. We imputed missing scores with the mean of the adjacent non-missing values. Prior to mean imputation, we analyzed cognitive growth trajectories and found cognitive scores remained stable or showed a consistent decline over follow-up, suggesting that mean imputation would not bias our results. However, the variability of the adjacent mean score to a given missing value may depend on time since baseline, thus the precision of the imputation may be reduced for imputed values further from cohort entry. After conducting latent class analyses without mean imputation, 21 participants were reclassified to the cognitive decline group and 4 participants were reclassified to the stable group, when compared to classification with mean imputation.

3.8 Sensitivity Analyses

We performed the following sensitivity analyses for which the results are detailed in the included manuscripts:

1. Excluding those with self-reported AF: In the REGARDS cohort, AF status was defined as evidence of AF on ECG or self-reported previous diagnosis. Therefore, participants self-reporting previous diagnosis of AF may have been misclassified, biasing our results. To account for this possible misclassification, we conducted a sensitivity analysis restricting

the cohort to those with ECG-confirmed AF. However, it is important to note that we were limited by a small sample size and those with intermittent AF may have been misclassified as non-AF cases by ECG.

2. Effect modification by sex and APOE genotype: We also hypothesized that the association between inflammation and cognitive outcomes may differ depending on sex and APOE genotype. To test whether sex and APOE genotype were effect modifiers on the multiplicative scale, sensitivity analyses were performed by separately entering sex and APOE Ɛ4 status as interaction terms in all models to assess the statistical significance of the estimated coefficient ($p<0.05$). If significant, we reported sex-specific estimates under the assumption that the covariates in the model are also modified by sex. If APOE Ɛ4 status was a significant effect modifier, we additionally reported the exponentiated sum of the estimated beta coefficients for the inflammatory marker and interaction term rather than reporting strata-specific estimates as we presumed the effect of the covariates would be the same across APOE Ɛ4 strata and to maintain statistical power.
3. Stratum-specific estimates by incident stroke: As outlined in our conceptual model, incident stroke was hypothesized as a potential mediator of the exposure-outcome relationship. To further investigate how incident stroke impacted the exposure-outcome relationship, we obtained stratum-specific estimates by incident stroke. This was particularly important for the REGARDS cohort, as more than half of the participants were recruited from the Stroke Belt region of the United States, a region with higher rates of stroke (125). We compared the sub cohort-specific estimates to each other and to our estimates in the overall cohort to check for confounding or effect modification by incident stroke.

4. Restricting the follow-up period to assess the impact of missingness: For analyses of cognitive trajectories in the REGARDS cohort, we reduced the follow-up period to approximately 8 years, as only 53% of the cohort had 8 consecutive cognitive assessments out of a maximum of 16. Upon further investigation, a large proportion of those with missing cognitive assessments were due to death prior to dementia (n = 786, 37%). To investigate whether this degree of missingness impacted our estimates, we ran a sensitivity analysis restricting the follow-up period to approximately 4 years (< 20% missingness).
5. Excluding those with MCI: While we excluded those with prevalent dementia in the CHS cohort, we retained those with MCI. As the cohort included 577 MCI cases and the study did not distinguish prevalent from incident cases, we did not exclude those with MCI as to not reduce our power. We instead conducted sensitivity analyses among the sub-cohort without MCI.
6. Secondary analyses without mean imputation: In the CHS study, we re-ran logistic regression models using the latent class analysis results in the absence of mean imputation. As few participants were reclassified, there were no significant changes to the estimates.
7. Secondary analyses with sex-specific classification: In the CHS study, we re-ran logistic regression models using the latent class analysis results generated within each sex strata. The sensitivity analyses showed no significant changes to the sex-specific estimates.

Chapter 4. Manuscript 1

Preface

The first manuscript addresses the following research objectives:

1. Estimate the associations between CRP and incident dementia in aging adults with documented AF.
2. Estimate the associations between CRP and cognitive decline in aging adults with documented AF.
3. Investigate these associations within subgroups of interest: by sex.

Ethics approval was granted from the University of Ottawa Heart Institute Research Ethics Board.

Inflammatory-mediated blood-brain barrier dysfunction and cognitive decline: a population-based cohort study among aging adults with atrial fibrillation

Danielle Marion[1], Zhe Li[2], Austyn Roseborough[3], Shawn N. Whitehead[3], Ellen E. Freeman[1,4], David H. Birnie[2], Fanny M. Elahi[5], Vladimir Hachinski[6], Jodi D. Edwards[1,2,*]

[1] School of Epidemiology and Public Health, University of Ottawa, Ottawa, ON, Canada

[2] University of Ottawa Heart Institute, Ottawa, ON, Canada

[3] Department of Anatomy & Cell Biology, University of Western Ontario, London, ON, Canada

[4] Ottawa Hospital Research Institute, Ottawa, ON, Canada

[5] Memory and Aging Center, Department of Neurology, University of California, San Francisco, California, United States

[6] Department of Clinical Neurological Sciences, London Health Sciences Centre, Western University, London, ON, Canada

DM contributed to the analyses, interpretation of the results, and drafting of the manuscript. ZL contributed to the analyses and revision of the manuscript. AR, SNW, EEF, DHB, FME, and VH contributed to the revision of the manuscript. JDE contributed to the data access, analyses, interpretation of the results, and revision of the manuscript.

*Corresponding Author: Jodi Edwards, jedwards@ottawaheart.ca, 613-696-7000 x 15336

Abstract

Background

Despite strong evidence for an association between atrial fibrillation (AF) and cognitive decline independent of stroke, contributing pathways underlying this relationship remain unclear. Critically, elevated levels of inflammatory markers, such as C-reactive protein (CRP), common in AF, are associated with increased blood-brain barrier (BBB) permeability, leaving the brain vulnerable to the influx of neurotoxic proteins that may contribute to neuroinflammation and neurodegeneration. To examine the role of these markers in dementia risk, we estimated associations of CRP with cognitive decline and dementia among aging adults with AF.

Methods

We conducted an analysis of adults aged ≥45 years with prevalent AF and without cognitive impairment from prospectively collected Reasons for Geographic and Racial Differences in Stroke (REGARDS) cohort. Plasma CRP levels were ascertained at baseline and cognitive status measured annually using the Six-Item Screener (SIS). Competing risks Cox proportional hazards regression was used to estimate cause-specific hazard for dementia, defined as an SIS score ≤ 3. Cognitive trajectories were identified using latent class growth models. Using adjusted binomial logistic regression, we estimated associations between CRP and cognitive trajectories and examined interactions by sex.

Results

Among 2,109 AF cases, 285 developed dementia and 786 died prior to the development of dementia over a median 9-year follow-up. Elevated CRP levels were associated with mortality (hazard ratio [HR]=1.13; 95% confidence interval [CI] 1.08-1.19) but not incident dementia (HR=0.98; 95% CI: 0.91-1.04). Latent class analyses identified two unique cognitive trajectories,

with 91% showing a stable trajectory of cognitive status and 9% showing progressive cognitive decline. Sex-specific models indicated that, although a two-fold increase in CRP levels increased the odds of having a progressive cognitive decline trajectory by 9% among males (odds ratio [OR]=1.09; 95% CI: 1.01-1.17), no association between CRP levels and cognitive trajectory was observed among females (OR=1.04; 95% CI: 0.97-1.12).

Conclusion

Elevated CRP was associated with cognitive decline in aging males with AF, highlighting inflammatory-mediated BBB dysfunction as a potential contributing pathway linking AF to cognitive decline and warranting further investigation to confirm causality.

Introduction

Atrial fibrillation (AF) and dementia are growing epidemics in the aging population, with a global prevalence projected to rise above 60 million and 139 million respectively by 2050 (1,2). AF, the most common cardiac arrhythmia, is associated with an increased risk of morbidity and mortality, increasing stroke risk 5-fold (3), and dementia is a leading contributor of disability and dependence among older adults (4). Critically, AF patients are at a higher risk of cognitive decline and dementia independent of stroke (5,6), but despite strong evidence supporting this association, the underlying pathways linking AF to cognitive decline remain poorly understood.

A potential contributing pathway of this association that has received relatively little attention in the clinical literature is the pro-inflammatory state common to both AF and dementia and its impact on the blood-brain barrier (BBB). Systemic inflammation in AF may increase BBB permeability which has been strongly related to cognitive decline and dementia (7). Increased BBB permeability subjects the brain to neurotoxins, activating central inflammatory responses, and impairs the clearance of amyloid beta (Aβ), leading to its accumulation and aggregation, and

resulting (7,8) in neurodegeneration, synaptic impairment, and neuronal damage associated with clinical dementia (7). Although elevated levels of inflammatory markers, including C-reactive protein (CRP) which is produced by the liver during the acute inflammatory response, have been associated with AF and BBB dysfunction (9–16), few studies have investigated associations between these markers and dementia in the AF population. CRP levels have been shown to increase in response to infection or inflammatory conditions, such as cardiovascular disease (CVD) (17). Since measuring BBB dysfunction is difficult in a clinical setting, the use of circulating inflammatory markers such as CRP, may provide insight into ongoing inflammatory processes that are known to influence BBB integrity. To examine the role of these markers in dementia risk, we estimated associations of plasma CRP with cognitive decline and dementia among aging adults with AF.

Methods

Data Source and Study Cohort

The Reasons for Geographic And Racial Differences in Stroke (REGARDS) study is a prospective population-based cohort study of 30,183 Black and White individuals aged ≥45 years in the United States (18). Participants were randomly sampled from commercially available nationwide lists of residents using mail and telephone contact between 2003 and 2007 (19). Eligible participants underwent a baseline telephone interview and an in-home visit to provide measurements of physical and cognitive status (18) and were contacted by telephone at 6-month intervals with retrieval of medical records over a maximum follow-up period of 17 years (18). Further details on the methods are available elsewhere (18).

The present study included participants with self-reported AF (n=2,163) or with evidence of AF on 12-lead electrocardiogram (ECG) (n=430). Those with baseline cognitive impairment

(n=2,557), defined as a Six-Item Screener (SIS) score ≤ 4 (20), or with missing CRP data (n=167) were excluded from this analysis. The REGARDS study was approved by Institutional Review Boards of participating institutions and all participants provided written informed consent. For the current study, ethics approval was granted by the University of Ottawa Heart Institute Research Ethics Board.

Study Exposure

The exposure was plasma CRP levels (mg/L) collected at the baseline in-home visit. Participants fasted 10 to 12 hours before the visit and blood samples were centrifuged at room temperature within 2 hours of collection (18). Separated serum and plasma samples were catalogued and re-centrifuged at 60,000 g and 4°C, then stored at the Central laboratory at -80°C (18). CRP was measured using high-sensitivity, particle enhanced immunonephelometric assay (N High Sensitivity CRP, Dade Behring Inc., Deerfield, IL; interassay coefficients of variation 2.1–5.7%) (21). CRP was $\log_2$ transformed to correct for skewness.

Outcomes

The primary outcome was incident dementia, ascertained by trained technicians at baseline and annually over follow-up using the SIS. The SIS assesses global cognitive function and includes a three-item recall and a three-item temporal orientation (day of the week, month, year) (20) with a score ranging from 0 to 6. Dementia was defined as a score of 3 or less at any follow-up assessment, a cut-off previously shown to have a sensitivity of 88.7% and specificity of 88.0% for the diagnosis of dementia (20). The secondary outcome was cognitive decline, indexed as trajectories of SIS scores over time.

Covariates

All models were adjusted for demographics, vascular risk factors, clinical comorbidities, and medication usage. These covariates were selected based on clinical rationale and evidence of their association with the exposure and outcome variables. Demographic covariates included self-reported age, sex, race, education level, and urban group. Education was categorized as less than high school, high school graduate, some college, or college graduate and above. Urbanicity was categorized as rural if the census tract where the participant lived was ≤ 25% urban, mixed if the census tract was 25-75% urban, or urban if the census tract was ≥75% urban. Vascular risk factors included self-reported smoking and body mass index (BMI). Smoking was defined as never, past, or current smoker. BMI (kg/m^2) was derived from measured height and weight at the baseline in-home visit. Clinical comorbidities included self-reported stroke at baseline, history of heart disease, diabetes, hypertension, and dyslipidemia. Participants were categorized as having a history of heart disease if they had reported previous myocardial infarction, coronary artery bypass graft, bypass, angioplasty or stenting, or if they had evidence of myocardial infarction on ECG. Diabetes was defined as a fasting glucose ≥ 126 mg/dL, a non-fasting glucose ≥ 200 mg/dL, or self-reported use of diabetic medication. Hypertension was defined as a systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥ 90 mm Hg recorded at the in-home visit. Dyslipidemia was defined as total cholesterol ≥ 240 mg/dL, low-density lipoprotein ≥ 160 mg/dL, high-density lipoprotein ≤ 40 mg/dL, or self-reported use of lipid lowering medication at baseline. Medications included self-reported use of antihypertensives, statins, antiplatelets, and anticoagulants during the baseline telephone interview. We also adjusted for SIS score recorded at baseline.

Statistical Analyses

Descriptive statistics were used to characterize the study cohort by dementia status with respect to demographic and clinical variables, using Chi-square tests, t-tests, or Wilcoxon Rank-Sum tests. Cohort characteristics are presented as frequencies and proportions for categorical variables and means or medians for continuous variables (with measures of variation, including standard deviation and interquartile range). We followed the Treatment And Reporting of Missing data in Observational Studies (TARMOS) framework to select the appropriate method for missing data (22). Based on these guidelines, we conducted complete case analyses, excluding records with missing covariate data and confirmed that multiple imputation would likely not improve efficiency using descriptive analyses exploring associations of missingness with exposure and outcome variables. Cumulative incidence function curves were generated to display the incidence of dementia and death over follow-up for those with low (<1.0 mg/L), moderate (1.0 – 3.0 mg/L), and high baseline CRP levels (>3.0 mg/L) (23).

For the primary analyses, Cause-specific competing risks Cox proportional hazards regression was used to estimate crude and adjusted hazard ratios for associations between CRP and incident dementia. A competing risks framework (24) was used to account for death as a competing risk between exposure and incident dementia. Time was parametrized as the number of days from the baseline in-home visit to the first occurrence of dementia, or to the last recorded cognitive assessment, or to the date of death prior to dementia, or loss to follow-up unrelated to death.

For secondary analyses, to limit missingness, we restricted follow-up to 8 cognitive assessments, the maximum number of cognitive assessments for which the proportion of missingness was < 50%. Cognitive trajectories were identified using latent class growth models, a

semiparametric approach that identifies distinct classes with similar outcome trajectories over time (25). The number of latent classes were selected based on class sizes and Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) fit indices. Binomial logistic regression was used to estimate associations between CRP and trajectory groups.

All models were adjusted for the above covariates. A multiplicative interaction term was used to determine effect modification by sex in all adjusted models, with strata-specific estimates estimated in the event of a statistically significant interaction ($p<0.05$). To ensure that inclusion of those with self-reported AF did not bias our results, we conducted a sensitivity analysis restricting the cohort to those with ECG-confirmed AF. To investigate the impact of incident stroke on model estimates, we conducted subgroup analyses among those both with and without incident stroke. Proportional hazards assumptions were assessed using Schoenfeld residual tests for each covariate and the global model ($p<0.05$) and visual inspection of plots of the scaled Schoenfeld residuals versus time. Plots of martingale residuals and logit of the outcome versus continuous covariates were visually inspected to detect non-linearity. To check for influential observations or outliers, we inspected plots of Dfbeta, Cook's distance, and deviance residuals. All analyses were conducted using SAS V.9.4 (SAS Institute, Inc. Cary, North Carolina, USA) and R V.4.0.3.

Results

A total of 2,109 adults with evidence of AF, without baseline cognitive impairment, and non-missing CRP values were included in the analytic cohort (Figure 4-4, Supplemental Material). Demographic and clinical characteristics of the cohort (overall and by dementia status) are presented in Table 4-1. Overall, the majority were White (65.6%) and female (55%). Individuals with dementia were significantly more likely to report using anticoagulants compared to those without dementia (29.1% versus 20.3%). Dementia cases were less likely to be female compared

to those without dementia (48.4% versus 56.1%). The median follow-up time was 9 years. Over the course of follow-up period, 285 individuals developed dementia and 786 individuals died prior to dementia incidence. In the adjusted models, 377 observations were deleted due to missingness.

Table 4-1. Characteristics of Study Cohort by Dementia Status

Characteristic	Overall (N=2,109)	No dementia (n=1,824)	Dementia (n=285)	p-value
Age, years (mean ± SD)	67.1 ± 9.6	66.3 ± 9.6	71.9 ± 8.1	0.09
Female, n (%)	1,161 (55.05)	1,023 (56.1)	138 (48.4)	<0.0001
Black race, n (%)	725 (34.4)	625 (34.3)	100 (35.1)	0.79
Education				0.23
Less than high school	251 (11.9)	207 (11.4)	44 (15.5)	
High school graduate	581 (27.6)	502 (27.6)	79 (27.8)	
Some college	574 (27.3)	501 (27.5)	73 (25.7)	
College graduate and above	699 (33.2)	611 (33.55)	88 (31.0)	
Income, n (%)				0.26
< $20,000	440 (20.9)	374 (20.5)	66 (23.2)	
$20,000-34,000 (or 999)	533 (25.3)	458 (25.1)	75 (26.3)	
$35,000-74,000 (999)	588 (27.9)	519 (28.45)	69 (24.2)	
≥ $75,000	275 (13.0)	244 (13.4)	31 (10.9)	
Refused to respond	273 (12.9)	229 (12.55)	44 (15.4)	
Has health Insurance	1,978 (93.4)	1,707 (93.7)	271 (95.1)	0.36
Urbanicity				0.41
Rural	211 (11.1)	187 (11.4)	24 (9.4)	
Mixed	229 (12.1)	193 (11.8)	36 (14.1)	
Urban	1,455 (76.8)	1,260 (76.8)	195 (76.5)	
Alcohol				0.86
Never	645 (30.6)	554 (30.4)	91 (31.9)	
Past	443 (21.0)	385 (21.1)	58 (20.35)	
Current	1,021 (48.4)	885 (48.5)	136 (47.7)	
Smoking				0.22
Never	876 (41.65)	756 (41.6)	120 (42.25)	
Past	953 (45.3)	817 (44.9)	136 (47.9)	
Current	274 (13.0)	246 (13.5)	28 (9.9)	
BMI, kg/m^2 (mean ± SD)	29.5 ± 6.4	29.6 ± 6.4	28.8 ± 6.5	0.06
Exercise				0.08
None	869 (41.8)	757 (42.1)	112 (40.0)	
1 to 3 times per week	667 (32.1)	587 (32.6)	80 (28.6)	
≥ 4 times per week	543 (41.8)	455 (25.3)	88 (31.4)	
Prior Medical History				

History of stroke, n (%)	194 (9.3)	159 (8.8)	35 (12.3)	0.06
Transient ischemic attack, n (%)	141 (7.5)	125 (7.6)	16 (6.5)	0.53
Hypertension, n (%)	122 (5.8)	111 (6.1)	11 (3.9)	0.13
Diabetes mellitus, n (%)	514 (24.5)	449 (24.7)	65 (23.0)	0.52
Dyslipidemia, n (%)	1,358 (65.0)	1,165 (64.6)	193 (68.0)	0.27
History of heart disease, n (%)	727 (35.3)	620 (34.9)	107 (38.1)	0.30
Baseline medication use				
Anticoagulants, n (%)	453 (21.5)	370 (20.3)	83 (29.1)	<0.001
Antihypertensives, n (%)	1,319 (65.3)	1,148 (65.8)	171 (62.0)	0.21
Statins, n (%)	818 (38.8)	711 (39.0)	107 (37.5)	0.64
Antiplatelets, n (%)	170 (8.1)	143 (7.8)	27 (9.5)	0.35
CRP, mg/L (median ± IQR)	2.6 ± 4.9	2.7 ± 5.0	2.4 ± 4.4	0.13

Chi-square tests were used for all categorical variables; t-tests were used for age and BMI by dementia status; a Wilcoxon rank sum test was used for CRP by dementia status due to a skewed distribution.

Primary Analyses: Incident dementia and death

Estimated cumulative incidence functions displaying incident dementia and death over follow-up categorized by CRP level are shown in Figure 4-1 and 4-2 below.

Figure 4-1. Cumulative incidence function curves for dementia by C-reactive protein level (low: < 1.0 mg/L, moderate: 1.0–3.0 mg/L and high: > 3.0 mg/L) over the course of survival time (years). The dotted lines around each curve represent the 95% confidence interval.

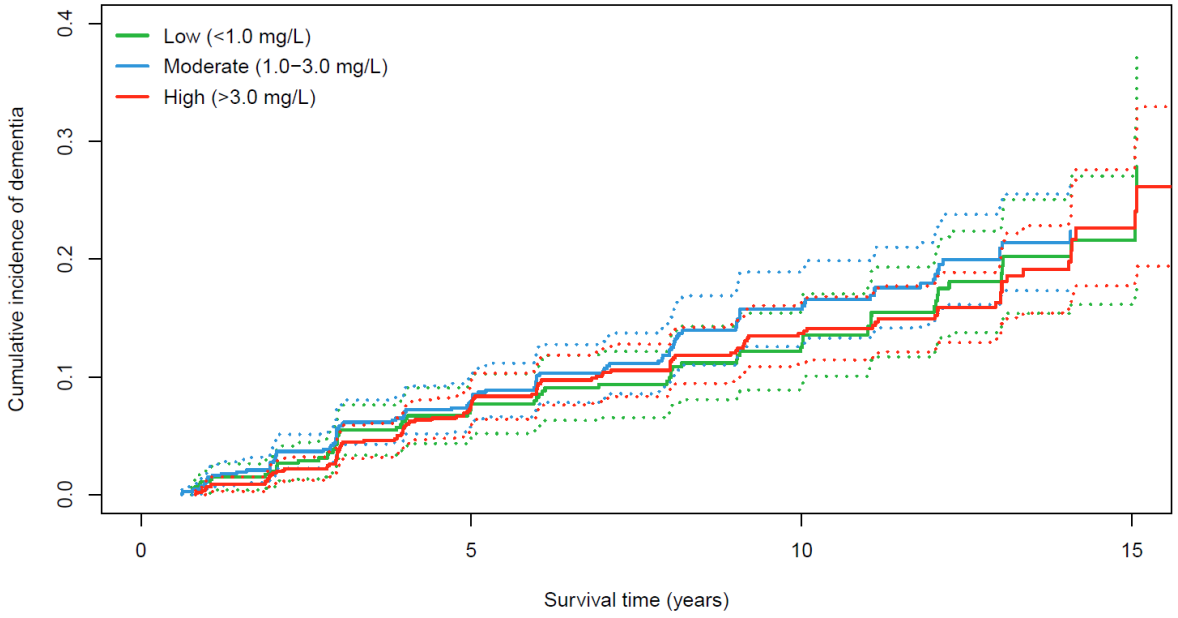
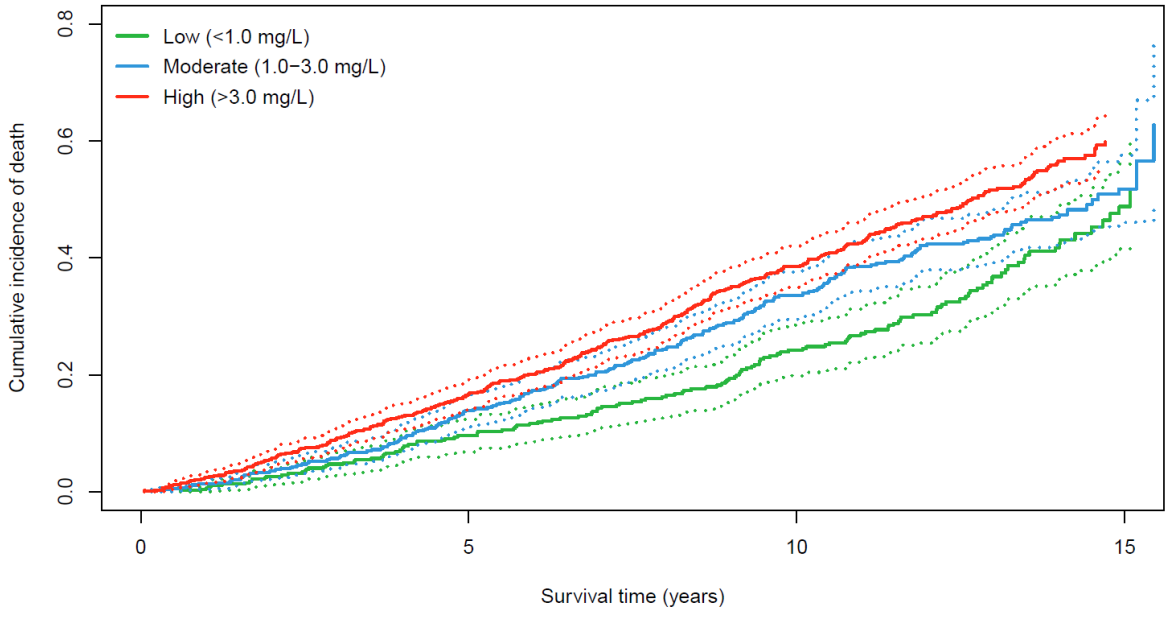


Figure 4-2. Cumulative incidence function curves for death by C-reactive protein level (low: < 1.0 mg/L, moderate: 1.0–3.0 mg/L and high: > 3.0 mg/L) over the course of survival time (years). The dotted lines around each curve represent the 95% confidence interval.



Estimated cause-specific hazards for primary outcomes of incident dementia and death are displayed in Table 4-2. After adjustment for age, sex, race, urban group, education, smoking, baseline cognitive score, BMI, baseline stroke, history of heart disease, diabetes, dyslipidemia, statins, antihypertensives, antiplatelets, and anticoagulants and death as a competing risk (dementia outcome only), a 2-fold increase in CRP did not significantly increase the cause-specific hazard of dementia (HR 0.97; 95% CI 0.90-1.06) but did increase the rate of death by 13% (95% CI 1.08-1.19). Based on the non-significance of the interaction term in the adjusted model ($p>0.9$), sex was not an effect modifier on the multiplicative scale and models were estimated in the overall cohort only.

Table 4-2. Cause-specific Cox proportional hazards model showing crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) between C-reactive protein (CRP) (mg/L) and incident dementia or death

	Dementia		**Death**	
	Crude HR (95% CI)	Adjusted* HR (95% CI)	Crude HR (95% CI)	Adjusted* HR (95% CI)
CRP (mg/L) (in logarithm base 2)	0.98 (0.91 – 1.04)	0.97 (0.90 – 1.06)	1.13 (1.08 – 1.17)	1.13 (1.08 – 1.19)

*Adjusting for age, sex, race, urban group, education, baseline SIS score, smoking, BMI, baseline stroke, history of heart disease, diabetes, hypertension, dyslipidemia, statins, antihypertensives, antiplatelets, and anticoagulants.

Secondary Analyses: Trajectories of Cognitive Decline Among Adults With AF

Latent class growth modeling indicated that data for AF patients were best modeled as two phenotypes with unique cognitive trajectories (Figure 4-3). The first phenotype, labeled “stable”, included 91% of the cohort and showed high SIS scores at baseline which remained stable over 15 years of follow-up. The second phenotype, labeled “cognitive decline”, included 9% of the cohort

and had high SIS scores at baseline that progressively declined over the 15-year follow-up. As sex was a significant effect modifier in the adjusted logistic regression model (p=0.013), we reported sex-specific estimates of cognitive decline (Table 4-3). A 2-fold increase in CRP significantly increased the odds of cognitive decline by 9% among males (OR 1.09; 95% CI 1.01 – 1.17), after covariate adjustment. However, there was no significant association between CRP and cognitive decline among females (OR 1.04; 95% CI 0.97 – 1.12), after adjustment for covariates.

Sensitivity Analyses

Sensitivity analyses restricting the cohort to those with ECG-confirmed AF and stratifying by incident stroke did not impact the primary findings. However, in secondary analyses, after exclusion of those without incident stroke, the association between CRP and cognitive decline remained significant only for those with incident stroke during follow-up (Supplemental).

Figure 4-3. Stable and declining trajectories of six-item screener (SIS) scores over time (years) among adults (aged 45+) identified and plot predicted using latent class growth modelling. The dotted lines around each trajectory represents the 95% confidence interval

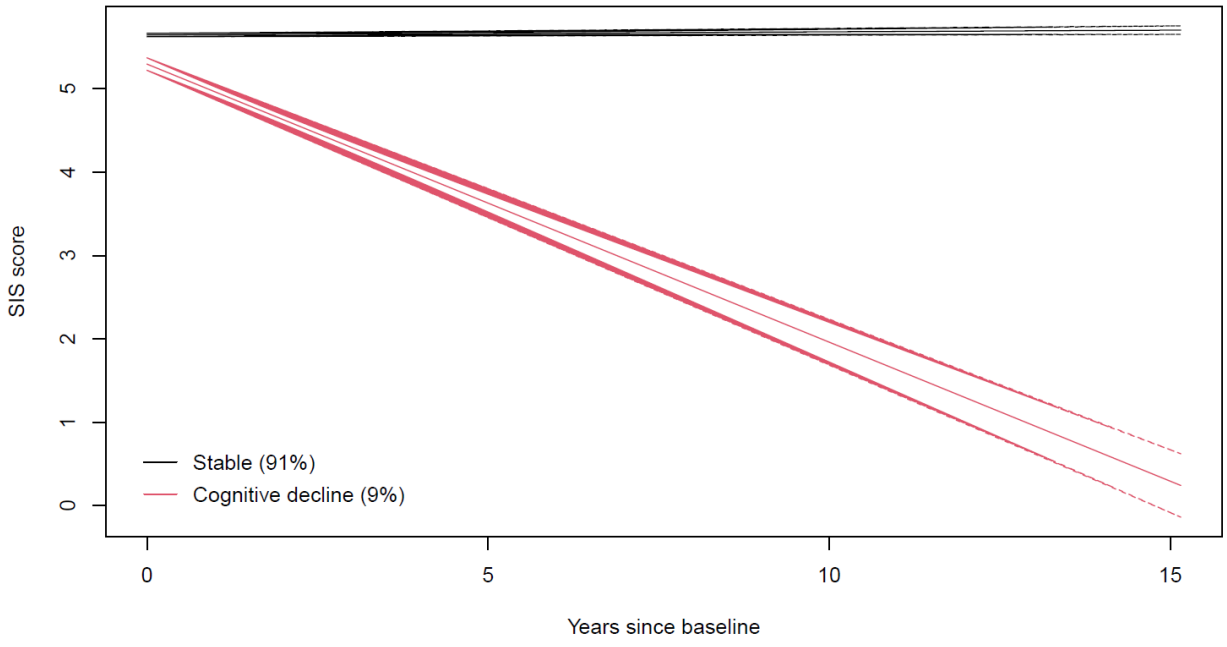


Table 4-3. Binomial logistic regression model showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between C-reactive protein (CRP) (mg/L) and cognitive decline relative to class 1 (Stable)

	Female-specific model		**Male-specific model**	
	Crude OR (95% CI)	Adjusted* OR (95% CI)	Crude OR (95% CI)	Adjusted* OR (95% CI)
CRP (mg/L) (in logarithm base 2)	0.96 (0.90 – 1.01)	1.04 (0.97 – 1.12)	1.16 (1.09 – 1.23)	1.09 (1.01 – 1.17)

*Adjusting for age, race, urban group, education, baseline SIS score, smoking, BMI, baseline stroke, history of heart disease, diabetes, hypertension, dyslipidemia, statins, antihypertensives, antiplatelets, and anticoagulants.

Discussion

In this longitudinal analysis of community-dwelling adults with AF, we found that elevated plasma levels of CRP, an inflammatory marker associated with CVD and BBB dysfunction (14–16), were associated with an increased rate of death, but not dementia after adjusting for demographic and clinical variables. Sex was not an effect modifier in adjusted models for incident dementia and death. Critically, we also showed that elevated CRP was associated with cognitive decline in aging males with AF.

To our knowledge, this study is the first to investigate the association between CRP and cognitive decline in an AF population. Previous studies have assessed the association between CRP and cognitive function in the general population. Among these, cross-sectional studies have typically reported significant associations between elevated CRP and cognitive impairment (26–30). However, longitudinal studies have had conflicting results (31–35). In another REGARDS study, higher CRP was associated with worse memory and verbal fluency at baseline but not

cognitive decline over follow-up of stroke-free adults at baseline (21). Others have reported that elevated CRP was associated with vascular dementia (VaD), but not Alzheimer's dementia (AD) or all-cause dementia (32,36). Although we were unable to investigate dementia subtypes, our results were consistent with studies reporting no association of elevated CRP with all-cause dementia. As VaD tends to progress more rapidly when compared to AD (37), a sufficient follow-up period may be required to detect symptoms of AD on neurocognitive batteries. Future studies should also consider investigating mixed pathologies, in which patients show signs of both AD and VaD, as it is increasingly recognized as the most common etiology of dementia in older adults (38–40).

Conflicting prior findings may also reflect variable demographics, comorbidity and APOE Ɛ4 prevalence, measures of cognition, and lengths of follow-up across studies. As dementia onset is typically after the age of 65 (37), sufficient follow-up is critical for younger cohorts. Our cohort had a mean age of 67 years and a median follow-up of 9 years. However, in the present study, dementia diagnosis relied on an SIS score ≤ 3. As the SIS is a brief cognitive screening instrument composed of only six simple cognitive tasks, it may result in a ceiling effect and a higher proportion of false negatives (41). Further, while this screener has been shown to be valid for dementia diagnoses, it does not involve all measures included in clinical criteria for dementia, such as comprehensive neuropsychological testing or assessment of activities of daily living. Domain-specific cognitive measures relating to dementia, including memory and executive function, may be more sensitive when compared to tests of global cognitive function and both a longer follow-up period and robust cognitive testing may have been required among individuals in this age stratum to detect dementia onset. For example, in a 25-year follow-up study with comprehensive neuroimaging and neuropsychological testing, higher CRP was associated with an increased risk

of all-cause dementia and dementia subtypes (34). However, this may be less important among an AF population, for whom are more likely to have a higher proportion of rapidly progressing VaD.

BBB dysfunction has previously been associated with cognitive decline and has been found in AF (42–46). Currently, it is difficult to directly measure BBB dysfunction or CNS inflammation in vivo, at scale, therefore circulating inflammatory markers such as interleukins or CRP have been widely used to measure ongoing inflammation. Systemic inflammation in AF may increase BBB permeability (8), leaving the brain vulnerable to the influx of neurotoxic plasma proteins and infiltration of subtypes of immune cells that may contribute to neuroinflammatory and neurodegenerative processes (42,47–49). Previous studies have provided evidence of BBB dysfunction related to cognitive impairment (50), irrespective of Aβ and tau deposition (43), and in post-mortem brain tissue of AD patients (51). Findings from the present study provide preliminary evidence in support of this pathway contributing to cognitive decline, particularly among males with AF. Future studies using more sensitive markers of central infiltration may provide greater insights into the role of the BBB in AF-dementia risk. For example, recent studies have highlighted the potential role of brain-derived extracellular vesicles (EVs) as non-invasive indicators of neuropathology (52). The ability to measure brain-derived EVs indicative of central inflammation and BBB dysfunction would compliment recent studies describing EVs, and their associated cargo, that are altered in AD, Frontotemporal dementia, and small vessel disease (53–56).

Sex-specific differences in circulating biomarkers of inflammation have been previously reported, where females had higher levels of CRP (57). Our study findings demonstrated that elevated CRP was associated with cognitive decline among males but not females. In post hoc analyses, females in the cohort were younger than males (65.8 ± 9.5 versus 68.6 ± 9.6,

respectively). However, females had a longer follow-up period when compared to males (9.2 years versus 8.2 years, respectively). This finding highlights potential sex-specific differences in inflammatory pathways contributing to cognitive decline. While males tend to score higher on visuospatial tasks, females tend to have higher scores on tests of verbal memory (58), such as the SIS, which may explain the higher proportion of males in the cohort with dementia compared to females. However, whether these sex-specific findings reflect discrepancies in study methodology or true biological differences remains unknown. Given evidence for widespread sex differences in both the concentrations and effects of multiple inflammatory markers on CVD risk (57), future work should continue to consider sex in the evaluation of inflammatory-mediated BBB dysfunction for dementia risk.

Further, whether BBB dysfunction contributes to dementia and dementia subtypes among those with AF remains unknown. As we found an association with cognitive decline but not dementia, CRP may be of greater value as an early biomarker of cognitive impairment. As dementia pathology progresses, CRP may begin to stabilize, limiting its predictive and discriminative utility. This tracks with evidence demonstrating cerebrospinal fluid and neuroimaging biomarkers sensitive to the onset and progression of cognitive impairment in dementia subjects (59). Given dementia's long preclinical stage, such biomarkers would be of value in clinical decision-making and lengthen the therapeutic window for intervention (59). Future prospective studies using sensitive markers of BBB dysfunction and comprehensive cognitive measures, while exploring potential sex-specific differences, are required.

The strengths of this study include a large population-based cohort with multiple cognitive assessments over a median follow-up time of 9 years. The prospective design allowed us to investigate temporal relationships between baseline CRP levels and cognitive decline, ruling out

reverse causality. We were also able to account for many confounding demographic and clinical variables with death as a competing risk. However, there were also several limitations. First, despite adjustment for many key confounders, we cannot discount the possibility of residual confounding. We lacked sufficient data on potential confounders, such as medication usage and APOE status and were also not able to examine effects by dementia subtype. Given prior findings that APOE Ɛ4 carriers with elevated CRP levels had the greatest risk of memory impairment (60) and that elevated CRP levels are associated with a lower risk of cognitive decline in those without the APOE Ɛ4 allele (35), potential interactions with APOE status in this analysis may have been missed. Second, we did not have information available on AF duration and subtype. Consequently, we were not able to identify those with the longest history or with persistent AF, who have the highest risk of dementia (61–65). Further, we were unable to confirm whether those with self-reported AF truly had the condition. As such, subjects with self-reported AF may have been misclassified, particularly given the low anticoagulant use at baseline. Third, we were limited to considering CRP levels at one fixed timepoint. CRP is an acute-phase protein where levels can vary over time and perhaps more so among those with AF. Although CRP levels have been shown to be relatively stable over a 1-year period (66), longitudinal analyses beyond this time may require repeated prospective measurements of CRP. Repeated plasma measurements would allow us to distinguish between elevated CRP levels reflective of acute inflammatory events or ongoing chronic peripheral inflammation. Furthermore, while elevated CRP levels reflect systemic inflammation, they may not accurately reflect levels of inflammation in the CNS. However, animal studies have more clearly delineated the pathways linking plasma CRP to BBB dysfunction (67). Finally, in secondary analyses, subjects with greater cognitive decline may have been more likely to be lost to follow-up, biasing our results towards the null.

Conclusion

Elevated levels of plasma CRP were associated with cognitive decline in aging males with AF. This study suggests that inflammatory-mediated BBB dysfunction may contribute to the risk of cognitive decline in AF populations. Future work would benefit from the use of sensitive markers of BBB dysfunction and central inflammation and comprehensive cognitive assessments, while considering possible sex-specific differences.

Sources of Funding

This research was supported by a grant from the Canadian Institutes of Health Research (PJT 169192).

Acknowledgments

This research project is supported by cooperative agreement U01 NS041588 co-funded by the National Institute of Neurological Disorders and Stroke (NINDS) and the National Institute on Aging (NIA), National Institutes of Health, Department of Health and Human Service. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NINDS or the NIA. Representatives of the NINDS were involved in the review of the manuscript but were not directly involved in the collection, management, analysis or interpretation of the data. The authors thank the other investigators, the staff, and the participants of the REGARDS study for their valuable contributions. A full list of participating REGARDS investigators and institutions can be found at: https://www.uab.edu/soph/regardsstudy/.

References

1. Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. Int J Stroke. 2020;1747493019897870–1747493019897870.
2. World Health Organization. Dementia [Internet]. [cited 2021 Nov 17]. Available from: https://www.who.int/news-room/fact-sheets/detail/dementia
3. Wolf PA, Abbott RD, Kannel WB. Atrial Fibrillation as an Independent Risk Factor for Stroke: The Framingham Study. Stroke. 1991 Aug;22(8):983–8.
4. Canadian Institute for Health Information. Dementia in Canada: Summary [Internet]. [cited 2021 Jan 30]. Available from: https://www.cihi.ca/en/dementia-in-canada/dementia-in-canada-summary
5. Kalantarian S, Stern TA, Mansour M, Ruskin JN. Cognitive Impairment Associated With Atrial Fibrillation. Ann Intern Med. 2013 Mar 5;158(5_Part_1):338–46.
6. Bailey MJ, Soliman EZ, McClure LA, Howard G, Howard VJ, Judd SE, et al. Relation of Atrial Fibrillation to Cognitive Decline (from the Reasons for Geographic and Racial Differences in Stroke [REGARDS] Study). Am J Cardiol. 2021 Jun 1;148:60–8.
7. Hussain B, Fang C, Chang J. Blood–Brain Barrier Breakdown: An Emerging Biomarker of Cognitive Impairment in Normal Aging and Dementia. Front Neurosci. 2021;15:978.
8. Aryal, Ritambhara, Patabendige, Adjanie. Blood–brain barrier disruption in atrial fibrillation: a potential contributor to the increased risk of dementia and worsening of stroke outcomes? Open Biol. 11(4).
9. Ding WY, Gupta D, Lip GYH. Atrial fibrillation and the prothrombotic state: revisiting Virchow's triad in 2020. Heart. 2020 Oct 1;106(19):1463–8.

10. Crandall MA, Horne BD, Day JD, Anderson JL, Muhlestein JB, Crandall BG, et al. Atrial Fibrillation and CHADS2 Risk Factors are Associated with Highly Sensitive C-Reactive Protein Incrementally and Independently. Pacing Clin Electrophysiol. 2009;32(5):648–52.

11. Anderson JL, Allen Maycock CA, Lappé DL, Crandall BG, Horne BD, Bair TL, et al. Frequency of elevation of C-reactive protein in atrial fibrillation. Am J Cardiol. 2004 Nov 15;94(10):1255–9.

12. Guo Y, Lip GYH, Apostolakis S. Inflammation in Atrial Fibrillation. J Am Coll Cardiol. 2012 Dec 4;60(22):2263–70.

13. Mukamal KJ, Tolstrup JS, Friberg J, Grønbaek M, Jensen G. Fibrinogen and Albumin Levels and Risk of Atrial Fibrillation in Men and Women (the Copenhagen City Heart Study). Am J Cardiol. 2006 Jul 1;98(1):75–81.

14. Hsuchou H, Kastin AJ, Mishra PK, Pan W. C-reactive protein increases BBB permeability: implications for obesity and neuroinflammation. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol. 2012;30(5):1109–19.

15. Rochfort KD, Collins LE, Murphy RP, Cummins PM. Downregulation of blood-brain barrier phenotype by proinflammatory cytokines involves NADPH oxidase-dependent ROS generation: consequences for interendothelial adherens and tight junctions. PloS One. 2014;9(7):e101815.

16. Elwood E, Lim Z, Naveed H, Galea I. The effect of systemic inflammation on human brain barrier function. Brain Behav Immun. 2017 May;62:35–40.

17. Ansar W, Ghosh S. Clinical significance of C-reactive protein. Singapore: Springer; 2020.

18. Howard VJ, Cushman M, Pulley L, Gomez CR, Go RC, Prineas RJ, et al. The reasons for geographic and racial differences in stroke study: objectives and design. Neuroepidemiology. 2005;25(3):135–43.

19. Howard VJ, Kleindorfer DO, Judd SE, McClure LA, Safford MM, Rhodes JD, et al. Disparities in stroke incidence contributing to disparities in stroke mortality. Ann Neurol. 2011;69(4):619–27.

20. Callahan CM, Unverzagt FW, Hui SL, Perkins AJ, Hendrie HC. Six-Item Screener to Identify Cognitive Impairment among Potential Subjects for Clinical Research. Med Care. 2002;40(9):771–81.

21. Arce Rentería M, Gillett SR, McClure LA, Wadley VG, Glasser SP, Howard VJ, et al. C-reactive protein and risk of cognitive decline: The REGARDS study. PloS ONE. 2020 Dec 31;15(12):e0244612.

22. Lee, Katherine J, Tilling, Kate M, Cornish, Rosie P, Little, Roderick J A, Bell, Melanie L, Goetghebeur, Els, et al. Framework for the treatment and reporting of missing data in observational studies: The Treatment And Reporting of Missing data in Observational Studies framework. J Clin Epidemiol. 2021 Jun 1 ;134 :79–88.

23. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. Markers of Inflammation and Cardiovascular Disease. Circulation. 2003 Jan 28;107(3):499–511.

24. Austin PC, Lee DS, Fine JP. Introduction to the Analysis of Survival Data in the Presence of Competing Risks. Circulation. 2016 Feb 9;133(6):601–9.

25. Wardenaar K. Latent Class Growth Analysis and Growth Mixture Modeling using R: A tutorial for two R-packages and a comparison with Mplus. [Internet]. PsyArXiv; 2020 [cited 2022 Jan 16]. Available from: https://psyarxiv.com/m58wx/

26. Kravitz BA, Corrada MM, Kawas CH. Elevated C-reactive protein levels are associated with prevalent dementia in the oldest-old. Alzheimers Dement J Alzheimers Assoc. 2009 Jul;5(4):318–23.

27. Watanabe Y, Kitamura K, Nakamura K, Sanpei K, Wakasugi M, Yokoseki A, et al. Elevated C-Reactive Protein Is Associated with Cognitive Decline in Outpatients of a General Hospital: The Project in Sado for Total Health (PROST). Dement Geriatr Cogn Disord EXTRA. 2016 Jan 19;6(1):10–9.

28. Wersching H, Duning T, Lohmann H, Mohammadi S, Stehling C, Fobker M, et al. Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. Neurology. 2010 Mar 30;74(13):1022–9.

29. Roberts RO, Geda YE, Knopman DS, Christianson TJH, Pankratz VS, Kullo IJ, et al. Association of C-reactive protein with mild cognitive impairment. Alzheimers Dement J Alzheimers Assoc. 2009 Sep;5(5):398–405.

30. Ravaglia G, Forti P, Maioli F, Brunetti N, Martelli M, Servadei L, et al. Serum C-Reactive Protein and Cognitive Function in Healthy Elderly Italian Community Dwellers. J Gerontol Ser A. 2005 Aug 1;60(8):1017–21.

31. Yaffe K, Lindquist K, Penninx BW, Simonsick EM, Pahor M, Kritchevsky S, et al. Inflammatory markers and cognition in well-functioning African-American and white elders. Neurology. 2003 Jul 8;61(1):76–80.

32. Hsu PF, Pan WH, Yip BS, Chen RCY, Cheng HM, Chuang SY. C-Reactive Protein Predicts Incidence of Dementia in an Elderly Asian Community Cohort. J Am Med Dir Assoc. 2017 Mar 1;18(3):277.e7-277.e11.

33. Dik MG, Jonker C, Hack CE, Smit JH, Comijs HC, Eikelenboom P. Serum inflammatory proteins and cognitive decline in older persons. Neurology. 2005 Apr 26;64(8):1371–7.

34. Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ. Early inflammation and dementia: A 25-year follow-up of the Honolulu-Asia aging study. Ann Neurol. 2002;52(2):168–74.

35. Lima TAS, Adler AL, Minett T, Matthews FE, Brayne C, Marioni RE, et al. C-reactive protein, APOE genotype and longitudinal cognitive change in an older population. Age Ageing. 2014 Mar 1;43(2):289–92.

36. Engelhart MJ, Geerlings MI, Meijer J, Kiliaan A, Ruitenberg A, van Swieten JC, et al. Inflammatory Proteins in Plasma and the Risk of Dementia: The Rotterdam Study. Arch Neurol. 2004 May 1;61(5):668–72.

37. Joseph F. Quinn. Dementia. John Wiley & Sons, Ltd; 2014.

38. Snyder HM, Corriveau RA, Craft S, Faber JE, Greenberg SM, Knopman D, et al. Vascular contributions to cognitive impairment and dementia including Alzheimer's disease. Alzheimers Dement. 2015 Jun 1;11(6):710–7.

39. Schneider, Julie A., Arvanitakis, Zoe, Bang, Woojeong, Bennett, David A. Mixed brain pathologies account for most dementia cases in community-dwelling older persons SYMBOL. Neurology. 69(24):2197–204.

40. James BD, Bennett DA, Boyle PA, Leurgans S, Schneider JA. Dementia From Alzheimer Disease and Mixed Pathologies in the Oldest Old. JAMA. 2012 May 2;307(17):1798–800.

41. De Roeck EE, De Deyn PP, Dierckx E, Engelborghs S. Brief cognitive screening instruments for early detection of Alzheimer's disease: a systematic review. Alzheimers Res Ther. 2019 Feb 28;11(1):21.

42. Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018 Mar;14(3):133–50.

43. Nation DA, Sweeney MD, Montagne A, Sagare AP, D'Orazio LM, Pachicano M, et al. Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction. Nat Med. 2019 Feb;25(2):270–6.

44. Cai Z, Qiao PF, Wan CQ, Cai M, Zhou NK, Li Q. Role of Blood-Brain Barrier in Alzheimer's Disease. J Alzheimers Dis. 2018 Jan 1;63(4):1223–34.

45. Zenaro E, Piacentino G, Constantin G. The blood-brain barrier in Alzheimer's disease. Neurobiol Dis. 2017 Nov;107:41–56.

46. Galenko O, Jacobs V, Knight S, Bride D, Cutler MJ, Muhlestein JB, et al. Circulating Levels of Biomarkers of Cerebral Injury in Patients with Atrial Fibrillation. Am J Cardiol. 2019 Dec 1;124(11):1697–700.

47. McGeer PL, Rogers J, McGeer EG. Inflammation, Antiinflammatory Agents, and Alzheimer's Disease: The Last 22 Years. J Alzheimers Dis JAD. 2016 Oct 4;54(3):853–7.

48. Holmes C. Review: systemic inflammation and Alzheimer's disease. Neuropathol Appl Neurobiol. 2013 Feb;39(1):51–68.

49. Koizumi K, Wang G, Park L. Endothelial Dysfunction and Amyloid-β-Induced Neurovascular Alterations. Cell Mol Neurobiol. 2016 Mar 1;36(2):155–65.

50. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-Brain Barrier Breakdown in the Aging Human Hippocampus. Neuron. 2015 Jan 21;85(2):296–302.

51. Ryu JK, McLarnon JG. A leaky blood–brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. J Cell Mol Med. 2009;13(9a):2911–25.

52. Badhwar A, Haqqani AS. Biomarker potential of brain-secreted extracellular vesicles in blood in Alzheimer's disease. Alzheimers Dement Diagn Assess Dis Monit. 2020;12(1):e12001.

53. Elahi FM, Harvey D, Altendahl M, Brathaban N, Fernandes N, Casaletto KB, et al. Elevated complement mediator levels in endothelial-derived plasma exosomes implicate endothelial innate inflammation in diminished brain function of aging humans. Sci Rep. 2021 Aug 10;11(1):16198.

54. Winston CN, Goetzl EJ, Akers JC, Carter BS, Rockenstein EM, Galasko D, et al. Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. Alzheimers Dement Diagn Assess Dis Monit. 2016;3(1):63–72.

55. Kapogiannis D, Mustapic M, Shardell MD, Berkowitz ST, Diehl TC, Spangler RD, et al. Association of Extracellular Vesicle Biomarkers With Alzheimer Disease in the Baltimore Longitudinal Study of Aging. JAMA Neurol. 2019 Nov 1 ;76(11) :1340–51.

56. Longobardi A, Benussi L, Nicsanu R, Bellini S, Ferrari C, Saraceno C, et al. Plasma Extracellular Vesicle Size and Concentration Are Altered in Alzheimer's Disease, Dementia With Lewy Bodies, and Frontotemporal Dementia. Front Cell Dev Biol [Internet]. 2021 [cited 2022 Apr 7];9. Available from: https://www.frontiersin.org/article/10.3389/fcell.2021.667369

57. Lau ES, Paniagua SM, Guseh JS, Bhambhani V, Zanni MV, Courchesne P, et al. Sex Differences in Circulating Biomarkers of Cardiovascular Disease. J Am Coll Cardiol. 2019 Sep 24;74(12):1543–53.

58. Andrew MK, Tierney MC. The puzzle of sex, gender and Alzheimer's disease: Why are women more often affected than men? Womens Health. 2018 Jan 1;14:1745506518817995.

59. Counts SE, Ikonomovic MD, Mercado N, Vega IE, Mufson EJ. Biomarkers for the Early Detection and Progression of Alzheimer's Disease. Neurotherapeutics. 2017 Jan 1;14(1):35–53.

60. Noble JM, Manly JJ, Schupf N, Tang MX, Mayeux R, Luchsinger JA. Association of C-Reactive Protein With Cognitive Impairment. Arch Neurol. 2010 Jan 1;67(1):87–92.

61. Bunch TJ, Weiss JP, Crandall BG, May HT, Bair TL, Osborn JS, et al. Atrial fibrillation is independently associated with senile, vascular, and Alzheimer's dementia. Heart Rhythm. 2010 Apr 1;7(4):433–7.

62. de Bruijn RFAG, Heeringa J, Wolters FJ, Franco OH, Stricker BHC, Hofman A, et al. Association Between Atrial Fibrillation and Dementia in the General Population. JAMA Neurol. 2015;72(11):1–7.

63. OTT A, BRETELER MMB, DE BRUYNE MC, VAN HARSKAMP F, GROBBEE DE, HOFMAN A. Atrial fibrillation and dementia in a population-based study: The Rotterdam study. Stroke 1970. 1997;28(2):316–21.

64. Kim D, Yang PS, Joung B. Prevention of Dementia in Patients with Atrial Fibrillation. Korean Circ J. 2021 Mar 11;51(4):308–19.

65. Stefansdottir H, Arnar DO, Aspelund T, Sigurdsson S, Jonsdottir MK, Hjaltason H, et al. Atrial fibrillation is associated with reduced brain volume and cognitive function independent of cerebral infarcts. Stroke. 2013 Apr;44(4):1020–5.

66. Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, Stanek E. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. Clin Chem. 2001 Mar;47(3):444–50.

67. Kuhlmann CRW, Librizzi L, Closhen D, Pflanzner T, Lessmann V, Pietrzik CU, et al. Mechanisms of C-Reactive Protein-Induced Blood–Brain Barrier Disruption. Stroke. 2009 Apr;40(4):1458–66.

Supplemental Materials

Figure 4-4. Study Cohort

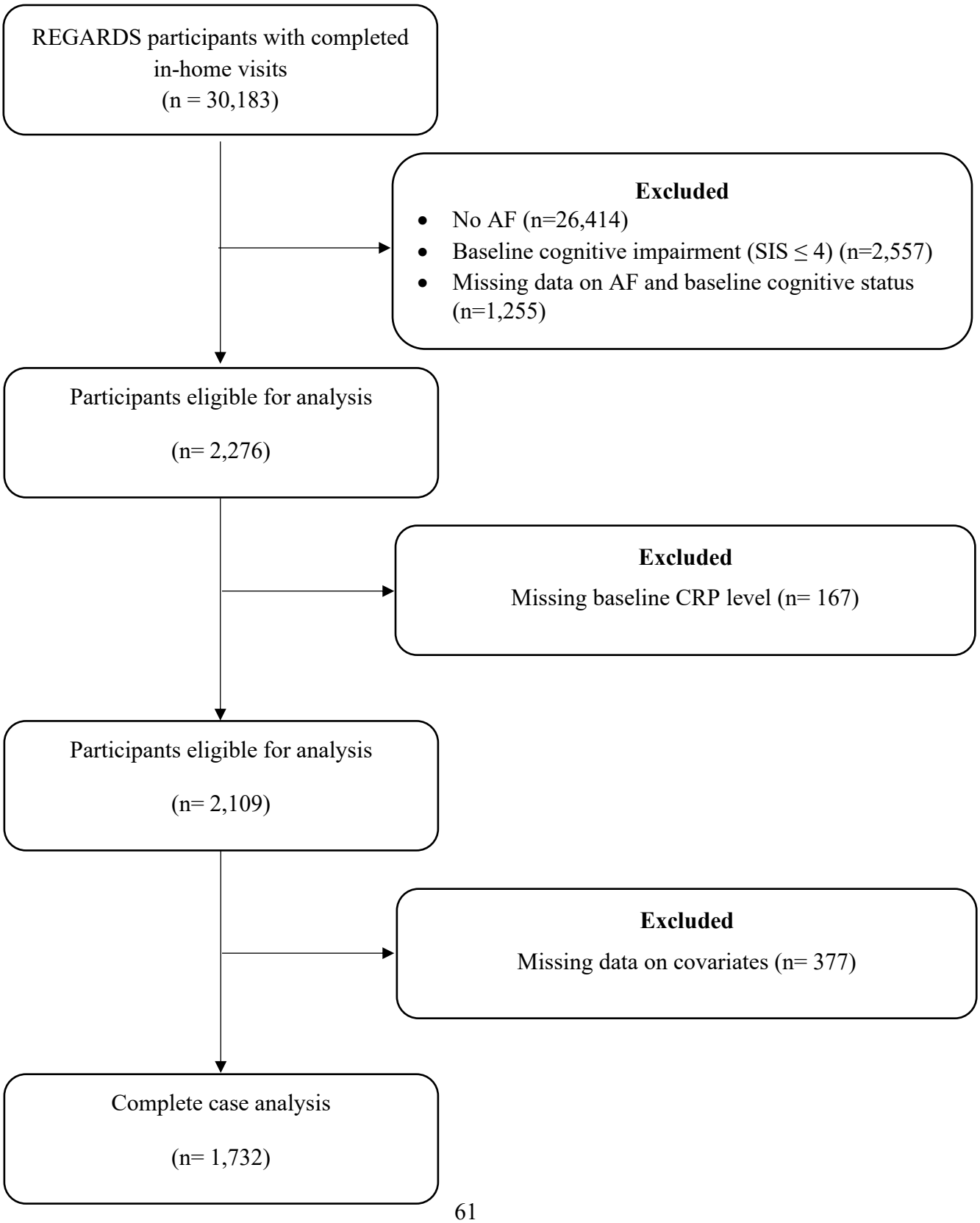


Table 4-4. Cause-specific Cox proportional hazards model showing crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) between C-reactive protein (mg/L) and incident dementia among sub-cohort with ECG-confirmed AF (n = 331)

	Crude HR (95% CI)	**Adjusted* HR (95% CI)**
CRP (mg/L) (in logarithm base 2)	1.03 (0.88-1.20)	1.18 (0.97-1.43)

*Adjusting for age, sex, race, urban group, education, baseline SIS score, smoking, BMI, baseline stroke, history of heart disease, diabetes, hypertension, dyslipidemia, statins, antihypertensives, antiplatelets, and anticoagulants.

Table 4-5. Cause-specific Cox proportional hazards model showing crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) between C-reactive protein (mg/L) and incident dementia among cohort, stratified by incident stroke status

	Without incident stroke (n = 1,881)		**With incident stroke (n = 228)**	
	Crude HR (95% CI)	Adjusted* HR (95% CI)	Crude HR (95% CI)	Adjusted* HR (95% CI)
CRP (mg/L) (in logarithm base 2)	0.96 (0.89-1.04)	0.94 (0.86-1.03)	1.04 (0.88-1.24)	1.18 (0.95-1.47)

*Adjusting for age, sex, race, urban group, education, baseline SIS score, smoking, BMI, baseline stroke, history of heart disease, diabetes, hypertension, dyslipidemia, statins, antihypertensives, antiplatelets, and anticoagulants.

Table 4-6. Binomial logistic regression model showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between C-reactive protein (CRP) (mg/L) and cognitive relative to class 1 (Stable) among sub-cohort with ECG-confirmed AF (n = 311)

	Crude OR (95% CI)	**Adjusted* OR (95% CI)**
CRP (mg/L) (in logarithm base 2)	0.98 (0.89-1.08)	1.03 (0.90-1.17)

*Adjusting for age, sex, race, urban group, education, baseline SIS score, smoking, BMI, baseline stroke, history of heart disease, diabetes, hypertension, dyslipidemia, statins, antihypertensives, antiplatelets, and anticoagulants.

Table 4-7. Binomial logistic regression model showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between C-reactive protein (CRP) (mg/L) and cognitive relative to class 1 (Stable), stratified by incident stroke status

	Without incident stroke (n = 1,745)		**With incident stroke (n = 207)**	
	Crude OR (95% CI)	Adjusted* OR (95% CI)	Crude OR (95% CI)	Adjusted* OR (95% CI)
CRP (mg/L) (in logarithm base 2)	1.00 (0.96-1.05)	1.02 (0.97-1.08)	1.14 (1.03-1.27)	1.45 (1.22-1.72)

*Adjusting for age, sex, race, urban group, education, baseline SIS score, smoking, BMI, baseline stroke, history of heart disease, diabetes, hypertension, dyslipidemia, statins, antihypertensives, antiplatelets, and anticoagulants.

Chapter 5. Manuscript 2

Preface

In the first manuscript we examined associations of CRP with incident cognitive decline and dementia among aging adults with AF. We also investigated effect modification of these associations by sex. While we addressed sub-aims of thesis objectives 1 through 3, the second manuscript addressed the following remaining objectives:

1. Estimate the associations between plasma-based markers of inflammation (including fibrinogen, CRP, and IL-6) and incident dementia in aging adults with documented AF.
2. Estimate the associations between plasma-based markers of inflammation (including fibrinogen, CRP, and IL-6) and cognitive decline and worsening white matter grade on MRI in aging adults with documented AF.
3. Investigate these associations within subgroups of interest: by sex and APOE genotype.

Ethics approval was granted from the University of Ottawa Heart Institute Research Ethics Board.

Unique cognitive trajectories associated with inflammatory markers of blood-brain barrier permeability in atrial fibrillation: An analysis of the Cardiovascular Health Study Cohort

Danielle Marion[1], Zhe Li[2], Austyn Roseborough[3], Shawn N. Whitehead[3], Ellen E. Freeman[1,4], David H. Birnie[2], Fanny M. Elahi[5], Vladimir Hachinski[6], Jodi D. Edwards[1,2,*]

[1] School of Epidemiology and Public Health, University of Ottawa, Ottawa, ON, Canada

[2] University of Ottawa Heart Institute, Ottawa, ON, Canada

[3] Department of Anatomy & Cell Biology, University of Western Ontario, London, ON, Canada

[4] Ottawa Hospital Research Institute, Ottawa, ON, Canada

[5] Memory and Aging Center, Department of Neurology, University of California, San Francisco, California, United States

[6] Department of Clinical Neurological Sciences, London Health Sciences Centre, Western University, London, ON, Canada

DM contributed to the analyses, interpretation of the results, and drafting of the manuscript. ZL contributed to the analyses and revision of the manuscript. AR, SNW, EEF, DHB, FME, and VH contributed to the revision of the manuscript. JDE contributed to the data access, analyses, interpretation of the results, and revision of the manuscript.

*Corresponding Author: Jodi Edwards, jedwards@ottawaheart.ca, 613-696-7000 x 15336

Abstract

Background

Despite evidence for associations between atrial fibrillation (AF) and cognitive decline independent of stroke, pathways underlying this relationship remain unclear. Elevated levels of peripheral inflammatory markers common in AF, are associated with increased blood-brain barrier (BBB) permeability, leaving the brain vulnerable to the influx of neurotoxic proteins that may contribute to neuroinflammation and neurodegeneration. To examine the role of these markers in dementia risk, we estimated associations of plasma C-reactive protein (CRP), interleukin-6 (IL-6), and fibrinogen with cognitive decline and dementia among aging adults both with and without AF.

Methods

We conducted an analysis of adults aged ≥ 65 years free of dementia from the prospective Cardiovascular Health Cognition Study. Plasma levels of CRP, IL-6, and fibrinogen were ascertained at baseline and follow-up. Dementia status was adjudicated by a committee of neurologists and psychiatrists and cognitive function was measured annually using the Modified Mini-Mental State Examination (3MS). Competing risks Cox proportional hazards regression was used to estimate associations between time-varying inflammatory markers and the onset of dementia. Cognitive trajectories were identified using latent class growth models. Using adjusted binomial logistic regression, we estimated associations between baseline inflammatory markers and cognitive trajectories. In all models, we examined associations in the overall cohort and stratified by AF status and examined interactions by sex and apolipoprotein E (APOE) Ɛ4 status.

Results

Among 3,375 adults (505 with AF and 2,870 without), 480 developed dementia over a median follow-up of 9.3 years. Among those with or without AF, no associations between inflammatory

markers and dementia were observed. Latent class analyses identified two unique cognitive trajectories, with 82% showing a stable trajectory of cognitive status and 18% showing progressive cognitive decline. Among those with AF, elevated CRP and IL-6 increased the odds of cognitive decline by 10% (95% CI 1.05 – 1.15) and 32% (95% CI 1.22 – 1.44), respectively. Sex-specific models indicated that although a 1 g/L increase in fibrinogen increased the odds of cognitive decline by 57% among females with AF (95% CI 1.36-1.81), no association was observed among males with AF (odds ratio [OR] 1.13, 95% CI 0.99-1.30). Elevated levels of IL-6 showed significant associations with cognitive decline in the non-AF cohort as well (OR 1.12, 95% CI 1.05 – 1.18).

Among those with AF, inflammatory markers were more positively associated with cognitive decline in the presence of the APOE Ɛ4 allele. However, in the non-AF sub-cohort, we detected qualitative interaction such that the inflammatory markers were positively associated with cognitive decline in the absence of the APOE Ɛ4 allele and not associated or negatively associated with cognitive decline in the presence of the APOE Ɛ4 allele.

Conclusion

While no significant associations were observed for dementia, elevated levels of IL-6 were associated with cognitive decline in aging adults both with and without AF. Further, elevated levels of CRP and fibrinogen were associated with cognitive decline among those with AF. Critically, sex and APOE genotype significantly modified these associations. Future work using more sensitive markers of central inflammation and BBB dysfunction are needed to confirm causality.

Introduction

Atrial fibrillation (AF) and dementia are growing epidemics of concern in the aging population. AF, the most common cardiac arrhythmia, has a global prevalence of over 37 million (1) and

dementia is a leading contributor of disability and dependence among older adults (2). AF independently increases the risk of stroke 5-fold (3), a well-known risk factor for dementia (4). However, there is increasing evidence for associations of AF with cognitive decline and dementia, independent of stroke (5). While various potential pathways underlying this association have been proposed (6), they remain unsubstantiated in clinical literature. A plausible contributing pathway linking AF to cognitive decline and dementia is inflammatory-mediated blood-brain barrier (BBB) dysfunction. A growing body of evidence demonstrates that inflammation is an important contributor to the initiation and perpetuation of AF (7–11) and to neurovascular and neurodegenerative changes in dementia (12–15). Systemic inflammation in AF may increase the permeability of the BBB which has been strongly related to cognitive decline and dementia (16). Increased BBB permeability leaves the brain vulnerable to the influx of neurotoxic blood-derived products into the central nervous system (CNS) (17). Evidence suggests that there is BBB dysfunction in the early stages of the Alzheimer's disease (AD) progression (17–20) and that it may be an important early biomarker of cognitive dysfunction (21,22). Further, a recent study showed that AF was associated with systemic biomarkers of BBB disruption and neuronal injury (23). Although elevated levels of inflammatory markers, including C-reactive protein (CRP), interleukin-6 (IL-6), and fibrinogen, have been associated with AF (7,9–11,24,25) and BBB dysfunction (26–32), few studies have investigated associations between these markers and dementia in the AF population.

IL-6 is a mediator of the acute phase inflammatory response and induces the hepatic synthesis of CRP and fibrinogen (33), and their levels have been shown to increase in response to infection or inflammatory conditions, such as cardiovascular diseases (CVD) (34–36). Since measuring BBB permeability is difficult in a clinical setting, the use of circulating inflammatory

markers may provide insight into ongoing inflammatory processes that are known to influence BBB integrity. The purpose of this study was to investigate this mechanistic link by estimating associations of plasma-based inflammatory markers (including CRP, IL-6, and fibrinogen) with incident cognitive decline and dementia among aging adults with versus without AF. We also examined effect modification of these associations by sex and by apolipoprotein E (APOE) Ɛ4 allele, a major genetic risk factor for AD, as previous population-based studies suggest that the effect of systemic inflammation on dementia and cognitive impairment vary according to these factors (37–40).

Methods

Data Source and Study Cohort

The Cardiovascular Health Study (CHS) was a prospective cohort study in 5,888 adults aged 65 or older (an original cohort of 5,201 in 1989-90 and a supplemental cohort of 687 predominantly Black participants in 1992-93) in four U.S. communities (41). Eligible participants were randomly sampled from Medicare eligibility lists in each area (41). Participants underwent baseline examinations and were followed through 1998-99 with annual clinical examinations, telephone interviews at 6-month intervals, and data were linked with Medicare claims (41). Cognitive function was evaluated annually using the Modified Mini-Mental State Examination (3MS) starting at year 3 (41). In 1998-99, an ancillary study, the Cardiovascular Health Cognition Study (CHCS), was initiated to classify dementia status among 3,608 CHS participants who agreed to have a brain MRI in 1991-94 and a cognitive test in the same year (42). Further details on the study design have been previously described (41). The present study included those from the CHCS and excluded those with prevalent dementia at baseline (n=3,375). The institutional review board at each center approved the study and written informed consent was obtained from all participants.

For the current study, ethics approval was granted by the University of Ottawa Heart Institute Research Ethics Board.

Exposures

The exposures included plasma CRP (mg/L), IL-6 (pg/mL), and fibrinogen (mg/dL). CRP and IL-6 were measured from blood samples collected at the baseline, year 5, and year 9. Fibrinogen was measured from blood samples collected at baseline and year 5. Participants fasted for 12 hours before the clinic visits (41). Plasma and serum samples were prepared and frozen at -70ºC and were shipped weekly on dry ice to the Central Blood Analysis Laboratory (41). CRP and IL-6 were measured using enzyme-linked immunosorbent assay (43). Fibrinogen was measured with a modification of the von Clauss method, using a BBL Fibrometer (41). All other details on laboratory methods have been previously described (44). In all analyses, CRP and IL-6 were $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Outcomes

The primary outcome was incident dementia. Detailed information regarding dementia classification has been previously described (42,45). In brief, using the data collected between 1989 and 1999, the participants from the CHCS were classified as high or low risk for dementia (45). At 3 of the sites, surviving high-risk White participants and all surviving Black participants were invited into the clinic for neuropsychological testing (45). At the remaining site, all surviving participants were evaluated regardless of risk status or race (45). A committee of neurologists and psychiatrists classified all participants based on the available data (42). Participants who did not meet dementia criteria but who presented cognitive deficits were classified with mild cognitive impairment (MCI) (42). If the date of dementia onset was before entry into the CHS, the participant

was classified with prevalent dementia (42). Six participants were not classified due to a lack of sufficient information (42).

The secondary outcome was cognitive decline, indexed as trajectories of 3MS scores over time. The 3MS assesses global cognitive function and extends the scope of the Mini-Mental State Examination, with a scoring range from 0 (worst) to 100 (best) (46). We also investigated worsening white matter grade (WMG) on MRI scans taken approximately 5 years apart (45). Based on a library of templates, scans were graded using a 10-point scale, ranging from 0 (no lesions) to 9 (most lesions) (47). Change in WMG was categorized into 3 levels: no change or decrease in grade, 1 grade increase, or 2 or more grade increase. Further details are reported in the Supplemental Materials attached.

Covariates

All models were adjusted for demographics, vascular risk factors, clinical comorbidities, and medication use. These covariates were selected based on clinical rationale and evidence of their association with the exposure and outcome variables (3,4,48–55). Demographic covariates included self-reported age, sex, race, and highest level of education attained. Vascular risk factors included body mass index (BMI), smoking status, and cholesterol levels. BMI (kg/m^2) was derived from measured height and weight at the baseline clinic examination. Smoking status was categorized as never, former, or current. Total cholesterol (mg/dL) was measured from blood samples collected at baseline.

Clinical comorbidities included baseline hypertension, diabetes mellitus, left ventricular hypertrophy, stroke, congestive heart failure, and myocardial infarction. Hypertension was categorized as normal, borderline, or hypertensive. Borderline was defined as a systolic blood pressure between 140 – 159 mm Hg or a diastolic blood pressure between 90 – 94 mm Hg.

Hypertensive was defined as a systolic blood pressure ≥ 160 mm Hg, or a diastolic blood pressure ≥ 95 mm Hg, or self-reported history of hypertension and use antihypertensive medication. Diabetes was defined as a fasting glucose ≥ 126 mg/dL or self-reported use of insulin or oral hypoglycemics. Left ventricular hypertrophy was defined as Minnesota Code 3 on baseline electrocardiogram (ECG) or sex-specific left ventricular mass on echocardiogram (males: >102 g/m2; females: >88 g/m2). Self-reported history of stroke was validated by review of medical records (56). To validate self-reported congestive heart failure, the use of both diuretics and either digitalis or vasodilators or confirmation via medical records was required (56). Self-reported history of myocardial infarction was validated by baseline ECG or review of medical records (56). Medication use was assessed by medication inventory. Medications included baseline use of anti-hypertensives and lipid-lowering medications. Use of anticoagulants and incident stroke were included as time-varying covariates in primary analyses.

Genotyping methods have been previously described (57). In brief, the 3 major allelic forms of the APOE gene (Ɛ2, Ɛ3, and Ɛ4) and its 6 genotypes were determined in the Core Molecular Genetics facility at the University of Vermont College of Medicine by the method of Hixson and Vernier (57,58).

Statistical Analyses

Descriptive statistics were used to characterize the study cohort overall and by AF status with respect to demographic and clinical variables, using Chi-square tests, Fischer's exact test, t-tests, or Wilcoxon Rank-Sum tests. Cohort characteristics were presented as frequencies and proportions for categorical variables and means or medians for continuous variables (with measures of variation, including standard deviation and interquartile range). We followed the Treatment And Reporting of Missing data in Observational Studies (TARMOS) framework to select the

appropriate method to handle the missing data (59). Based on these guidelines, we conducted complete case analyses, excluding records with missing exposure and covariate data.

For the primary analyses, Cause-specific competing risks Cox proportional hazards regression was used to estimate crude and adjusted hazard ratios for associations between time-varying inflammatory markers and incident dementia. A competing risks framework (60) was used to account for death as a competing risk between exposure and incident dementia. Time was parametrized as the number of days from study entry to dementia diagnosis, or to the date of death, or to loss to follow-up unrelated to death, or to the end of the follow-up period (June 30, 1999).

For secondary analyses, cognitive trajectories were identified using latent class growth models, a semiparametric approach that identifies distinct classes with similar outcome trajectories over time (61). Assessment of cognitive decline began at year 3, when the 3MS was first administered, and was parametrized as time 0. Missing cognitive scores were imputed with the mean of the adjacent non-missing values. The number of latent classes were selected to ensure large enough class sizes while minimizing the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) fit indices. Binomial logistic regression was used to estimate associations between baseline inflammatory markers and trajectory groups. For exploratory analyses of worsening WMG, the cohort was limited to those in the CHCS with two MRI scans. Multinomial logistic regression was used to estimate crude and adjusted odds ratios for associations between baseline inflammatory markers and worsening WMG (results reported in the Supplemental materials attached).

For all analyses, estimates were generated for the overall cohort and stratified by AF status (including both prevalent and incident cases). AF status was defined by ECG or self-report of a physician's diagnosis of this condition (41). In primary analyses, AF was defined as those with

prevalent AF or incident AF prior to the development of dementia. To account for possible confounding, all models were adjusted for the above covariates. A multiplicative interaction term was used to determine effect modification by sex and APOE genotype in all adjusted models. In the event of statistically significant interaction ($p<0.05$), sex-specific models were estimated. In the event of interaction by APOE genotype, we additionally reported the exponentiated sum of the estimated beta coefficients for the inflammatory marker and interaction term. For sensitivity analyses we conducted two subgroup analyses to assess the impact on model estimates: the first, excluding those with MCI and the second excluding those with incident stroke.

Proportional hazards assumption were assessed using Schoenfeld residual tests for each covariate and the global model ($p<0.05$) and visual inspection of plots of the scaled Schoenfeld residuals versus time. Plots of martingale residuals and logit of the outcome versus continuous covariates were visually inspected to detect non-linearity. To check for influential observations or outliers, we inspected plots of Dfbeta, Cook's distance, and deviance residuals. All analyses were conducted using SAS V.9.4 (SAS Institute, Inc. Cary, North Carolina, USA) and R V.4.0.3.

Results

Characteristics of the Study Cohort

A total of 3,375 adults from the CHCS without prevalent dementia were included in the analytic cohort, 505 with AF and 2,870 without. Demographic and clinical characteristics of the cohort (overall and by AF status) are presented in Table 5-1. Overall, the majority of participants were white (85.0%) and female (59.1%) with a mean age of 71.9 years. Compared to those without AF, those with AF were more likely to be older (73.1 ± 5.1 versus 71.7 ± 4.7), have a prior history of stroke (5.7% versus 2.3%), transient ischemic attack (TIA) (5.2% versus 1.7%), congestive heart failure (6.1% versus 1.7%), and myocardial infarction (12.7% versus 6.5%). They were also more

likely to have diabetes (16.5% versus 12.2%), hypertension (46.5% versus 39.8%), left ventricular hypertrophy (5.7% versus 3.3%), use anticoagulants (3.6% versus 0.5%), use antihypertensives (52.5% versus 41.8%), and have higher levels of CRP (2.7 ± 3.4 versus 2.3 ± 2.7) and IL-6 (1.9 ± 1.4 versus 1.5 ± 1.2), compared to those without AF. They were less likely to be female (46.9% versus 61.2%) and Black (8.5% versus 15.6%) and had lower levels of cholesterol (203.1 ± 34.1 versus 212.8 ± 38.7), compared to those without AF. Over a median follow-up of 9.3 years, 480 (14%) individuals developed dementia and 2,479 (73%) died prior to any evidence of dementia.

Table 5-1. Characteristics of the CHCS study cohort overall and by AF status

Characteristic	Overall (N=3,375)	Without AF (n=2,870)	AF (n=505)	p-value
Age, years (mean ± SD)	71.9 ± 4.8	71.7 ± 4.7	73.1 ± 5.1	<0.0001
Female, n (%)	1,994 (59.1)	1,757 (61.2)	237 (46.9)	<0.0001
Black race, n (%)	492 (14.6)	449 (15.6)	43 (8.5)	<0.0001
Education				0.57
High school or less	251 (11.9)	1,504 (52.5)	272 (53.9)	
More than high school	699 (33.2)	1,361 (47.5)	233 (46.1)	
Smoking				0.30
Never	1,605 (47.6)	1,376 (48.0)	229 (45.4)	
Former	1,401 (41.6)	1,176 (41.0)	225 (44.6)	
Current	365 (10.8)	315 (11.0)	50 (9.9)	
BMI, kg/m^2 (mean ± SD)	26.5 ± 4.4	26.5 ± 4.4	26.8 ± 4.4	0.10
Cholesterol, mg/dL (mean ± SD)	211.4 ± 38.2	212.8 ± 38.7	203.1 ± 34.1	<0.0001
Medical History, n (%)				
History of stroke	94 (2.8)	65 (2.3)	29 (5.7)	<0.0001
Transient ischemic attack	76 (2.3)	50 (1.7)	26 (5.2)	<0.0001
Left ventricular hypertrophy	120 (3.7)	92 (3.3)	28 (5.7)	0.01
Hypertension				0.01
Normal	1,520 (45.1)	1,321 (46.1)	199 (39.4)	
Borderline	477 (14.2)	406 (14.2)	71 (14.1)	
Hypertensive	1,375 (40.8)	1,140 (39.8)	235 (46.5)	
Diabetes mellitus	427 (12.8)	344 (12.2)	83 (16.5)	0.01
Congestive heart failure	79 (2.3)	48 (1.7)	31 (6.1)	<0.0001
Myocardial infarction	249 (7.4)	185 (6.5)	64 (12.7)	<0.0001
Baseline medication use, n (%)				
Anticoagulants	32 (1.0)	14 (0.5)	18 (3.6)	<0.0001
Antihypertensives	1,463 (43.4)	1,198 (41.8)	265 (52.5)	<0.0001
Lipid-lowering medication	177 (5.3)	149 (5.2)	28 (5.5)	0.75
APOE Ɛ4, n (%)	745 (24.2)	635 (24.3)	110 (23.5)	0.72

Baseline CRP, mg/L (median ± IQR)	2.3 ± 2.9	2.3 ± 2.7	2.7 ± 3.4	0.004
Baseline IL-6, pg/mL (median ± IQR)	1.6 ± 1.2	1.5 ± 1.2	1.9 ± 1.4	<0.0001
Baseline fibrinogen, mg/dL (mean ± SD)	317.0 ± 62.5	316.7 ± 62.5	318.6 ± 62.6	0.54

Chi-square tests were used for all categorical variables with the exception of anticoagulant use, where a Fischer's exact test was used; t-tests were used for age, BMI, cholesterol, and fibrinogen by AF status; a Wilcoxon rank sum test was used for CRP and IL-6 by AF status due to skewed distributions.

Missing values (n): smoking = 4, BMI = 9, cholesterol = 20, left ventricular hypertrophy = 96, hypertension = 3, diabetes = 44, anticoagulants = 6, antihypertensives = 6, lipid-lowering medication = 6, APOE = 291, CRP = 33, IL-6 = 261, fibrinogen = 37.

Primary Analyses: Incident Dementia

Estimated cause-specific hazards ratios for incident dementia and death are displayed in Table 5-2. Based on the non-significance of the introduced interaction terms, sex was not an effect modifier on the multiplicative scale. After adjustment for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, antihypertensive use, lipid-lowering medication use, and time-varying adjustment for incident stroke and anticoagulant use, and accounting for death as a competing risk (dementia outcome only), an increase in any of the three inflammatory markers did not significantly increase the cause-specific hazard of dementia or death among those with AF.

Among those without AF and in the overall cohort, an increase in any of the three inflammatory markers did not significantly increase the cause-specific hazard of dementia after adjustment for the same covariates. However, elevated levels of all three inflammatory markers significantly increased the rate of death. Further, APOE genotype was a significant effect modifier of the relationship between IL-6 and dementia (interaction term p-value=0.015). While the presence of the APOE Ɛ4 allele appeared to attenuate the relationship between IL-6 and dementia, the association was not significant (HR 0.88, 95% CI 0.70-1.11). Sensitivity analyses excluding

those with MCI (n = 577) or incident stroke (n = 671) did not impact the observed estimates. Results from sensitivity analyses are displayed in the attached Supplemental Materials.

Table 5-2. Cause-specific Cox proportional hazards model showing crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) between inflammatory markers and incident dementia or death

Cohort	Time-varying exposure†	Dementia HR (95% CI)		Death HR (95% CI)	
		Crude	Adjusted*	Crude	Adjusted*
Adults with AF prior to incident dementia (n=505)	CRP	1.03 (0.88-1.20)	0.98 (0.84-1.15)	1.00 (0.94-1.06)	0.98 (0.92-1.05)
	IL-6	1.13 (0.85-1.51)	0.93 (0.67-1.29)	1.19 (1.06-1.34)	1.12 (0.99-1.28)
	Fibrinogen	0.99 (0.69-1.44)	0.91 (0.61-1.36)	1.04 (0.91-1.19)	1.01 (0.87-1.17)
Adults without AF (n=2,870)	CRP	0.96 (0.90-1.02)	0.96 (0.90-1.03)	1.03 (1.00-1.06)	1.04 (1.01-1.07)
	IL-6	1.25 (1.12-1.40)	1.11 (0.98-1.26)	1.19 (1.13-1.25)	1.16 (1.09-1.22)
	IL-6*APOE Ɛ4	1.07 (0.87-1.31)	0.88 (0.70-1.11)		
	Fibrinogen	1.22 (1.06-1.41)	1.09 (0.93-1.27)	1.16 (1.08-1.24)	1.09 (1.02-1.18)
Overall cohort (n=3,375)	CRP	0.97 (0.91-1.02)	0.97 (0.91-1.03)	1.03 (1.00-1.06)	1.03 (1.00-1.06)
	IL-6	1.23 (1.11-1.36)	1.08 (0.97-1.21)	1.20 (1.15-1.26)	1.15 (1.09-1.21)
	IL-6*APOE Ɛ4	1.04 (0.86-1.27)	0.87 (0.70-1.08)		
	Fibrinogen	1.18 (1.04-1.35)	1.06 (0.92-1.23)	1.15 (1.08-1.22)	1.09 (1.02-1.16)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, incident stroke (time-varying), left ventricular hypertrophy, congestive hart failure, myocardial infarction, antihypertensives, lipid-lowering medication, and anticoagulant use (time-varying).

†CRP and IL-6 were $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Secondary Analyses: Trajectories of Cognitive Decline Among Adults with and without AF

Latent class growth modeling indicated that data were best modeled as two phenotypes with unique cognitive trajectories (Figure 5-1). The first phenotype, labeled “stable”, included 82% of the cohort (n=2,773) and showed high 3MS scores at baseline which remained stable over follow-up.

The second phenotype, labeled “cognitive decline”, included 18% (n=602) of the cohort and had 3MS scores that progressively declined over follow-up.

Figure 5-1. Stable and declining trajectories of Modified Mini-Mental State Examination (3MS) scores over time (years) among adults (aged 65+) identified and plot predicted using latent class growth modelling. The dotted lines around each trajectory represents the 95% confidence interval

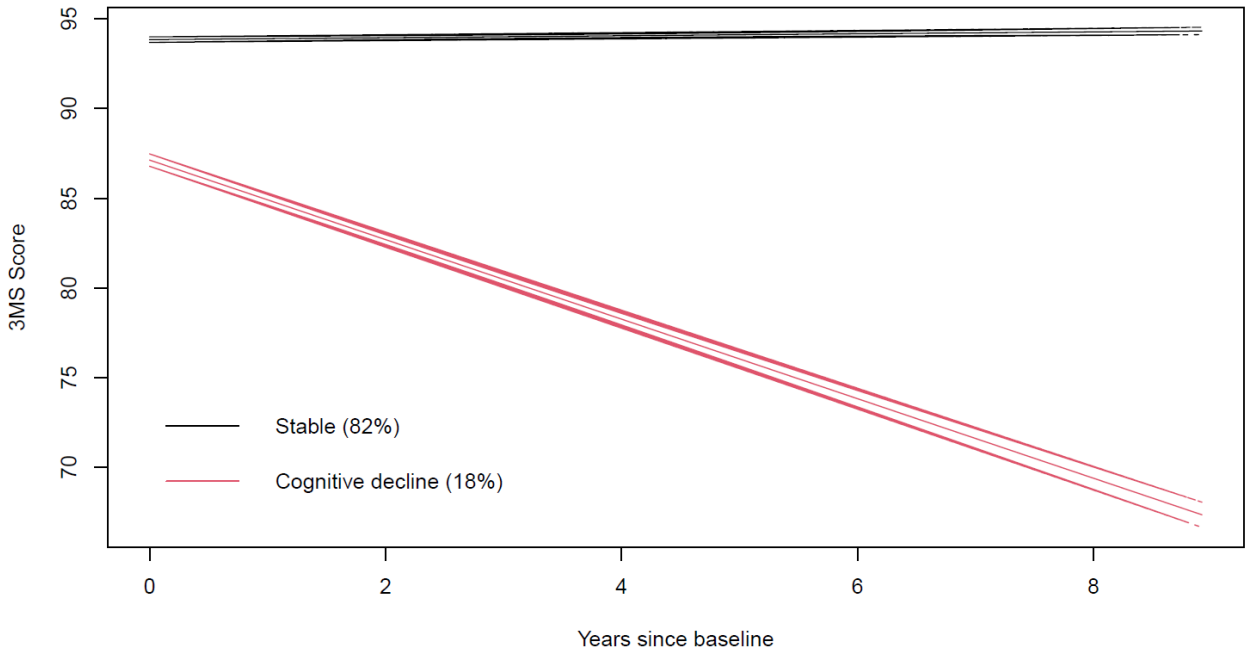


Table 5-3 presents the results from the logistic regression models for cognitive decline where sex was not a significant effect modifier. After adjustment for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, and baseline medication use (antihypertensives, lipid-lowering medications, and anticoagulants), a 2-fold increase in baseline IL-6 increased the odds of cognitive decline by 32% (95% CI 1.22-1.44) among those with AF. We found that APOE genotype significantly modified the effect of CRP on cognitive decline (interaction term p-value <0.001). For those without the APOE Ɛ4 allele, a 2-fold increase in CRP increased the odds of cognitive

decline by 10% (95% CI 1.05 – 1.15). Whereas, in the presence of the APOE Ɛ4 allele the effect of CRP on cognitive decline was more pronounced (OR 1.31, 95% CI 1.19-1.45).

Among those without AF, APOE genotype was also a significant effect modifier of the association between IL-6 and cognitive decline (interaction term p-value <0.001), however we found qualitative interaction as it had the opposite effect compared to those with AF. In non-AF participants without the APOE Ɛ4 allele, a 2-fold increase in IL-6 increased the odds of cognitive decline by 12% (95% CI 1.05-1.18). However, this association was attenuated and non-significant in the presence of the APOE Ɛ4 allele (OR 0.92, 95% CI 0.82-1.03). No significant association was observed between fibrinogen and cognitive decline among those without the APOE Ɛ4 allele. However, in the presence of the APOE Ɛ4 allele, a 1 g/L increase in fibrinogen decreased the odds of cognitive decline by 23%. In sensitivity analyses excluding those with MCI or incident stroke, associations with IL-6 in both cohorts and with CRP in the AF cohort with the APOE Ɛ4 allele remained significant (see Supplemental Materials attached).

Table 5-3. Binomial logistic regression model showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between inflammatory markers and cognitive relative to class 1 (Stable)

Cohort	Baseline Exposure†	Class 2: Cognitive Decline OR (95% CI)	
		Crude	Adjusted*
Adults with prevalent or incident AF (n=1,174)	CRP	1.11 (1.06-1.15)	1.10 (1.05-1.15)
	CRP*APOE Ɛ4	1.36 (1.25-1.49)	1.31 (1.19-1.45)
	IL-6	1.33 (1.24-1.42)	1.32 (1.22-1.44)
Adults without AF (n=2,201)	IL-6	1.29 (1.23-1.35)	1.12 (1.05-1.18)
	IL-6*APOE Ɛ4	1.11 (1.00-1.23)	0.92 (0.82-1.03)
	Fibrinogen	1.16 (1.09-1.24)	0.97 (0.90-1.05)
	Fibrinogen*APOE Ɛ4	0.94 (0.82-1.09)	0.77 (0.66-0.90)
Overall cohort (n=3,375)	CRP	1.09 (1.06-1.11)	1.04 (1.02-1.07)
	IL-6	1.30 (1.25-1.35)	1.17 (1.12-1.23)
	IL-6*APOE Ɛ4	1.24 (1.14-1.35)	1.05 (0.95-1.15)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, and baseline use of antihypertensives, lipid-lowering medications and anticoagulants.

†CRP and IL-6 were $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Secondary Analyses: Sex-specific Effects

Sex was a significant effect modifier in the adjusted logistic regression models for fibrinogen in the AF and overall cohort and for CRP in the non-AF cohort. Sex-specific estimates are reported in Table 5-4. While we found a positive association between fibrinogen and cognitive decline among females with AF (OR 1.57, 95% CI 1.36-1.81), no significant association was found in males. Further, we found significant effect modification by APOE Ɛ4 status, where the observed association was significantly stronger in the presence of the APOE Ɛ4 allele (OR 2.27, 95% CI 1.71-3.00).

In the overall cohort, a 1 g/L increase in fibrinogen increased the odds of cognitive decline by 17% among females (95% CI 1.08-1.27). Whereas, among males without the APOE Ɛ4 allele, a 1 g/L fibrinogen did not significantly increase the odds of cognitive decline (OR 1.03, 95% CI 0.94-1.13). However, in the presence of the APOE Ɛ4 allele, fibrinogen was negatively associated with cognitive decline (OR 0.74, 95% CI 0.61-0.89). These associations remained consistent in sensitivity analyses excluding those with MCI or incident stroke (see Supplemental Materials attached).

Table 5-4. Sex-specific binomial logistic regression models showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between inflammatory markers and cognitive relative to class 1 (Stable)

Cohort	Exposure†	Class 2: Cognitive Decline OR (95% CI)			
		Female-specific model		Male-specific model	
		Crude	Adjusted*	Crude	Adjusted*
Adults with prevalent or incident AF (n=1,174)	Fibrinogen	1.62 (1.43-1.83)	1.57 (1.36-1.81)	1.20 (1.07-1.35)	1.13 (0.99-1.30)
	Fibrinogen*APOE Ɛ4	2.59 (2.00-3.35)	2.27 (1.71-3.00)		
Adults without AF (n=2,201)	CRP	1.11 (1.07-1.15)	1.04 (1.00-1.09)	1.04 (0.99-1.09)	0.96 (0.90-1.01)
Overall cohort (n=3,375)	Fibrinogen	1.36 (1.27-1.45)	1.17 (1.08-1.27)	1.11 (1.03-1.20)	1.03 (0.94-1.13)
	Fibrinogen*APOE Ɛ4			0.95 (0.79-1.10)	0.74 (0.61-0.89)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, and baseline use of antihypertensives, lipid-lowering medications and anticoagulants.

†CRP was $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Discussion

This study demonstrated no significant associations of CRP, IL-6, and fibrinogen with dementia among those with AF or without AF. In those with AF, we observed positive associations of CRP, IL-6, and fibrinogen with cognitive decline. Fibrinogen displayed a sex-specific effect where the association was only significant in AF females. Among those without AF, we observed positive associations of IL-6 with cognitive decline. Further, effect modification by APOE Ɛ4 status had an opposite effect in the AF subgroup compared to the non-AF subgroup. Where the presence of

APOE Ɛ4 allele increased the observed associations in those with AF, they were attenuated in the non-AF cohort.

Previous studies have investigated associations of these inflammatory markers with cognitive decline and dementia in the general population, irrespective of AF status. Among them, cross-sectional studies have typically reported significant associations of elevated CRP, IL-6 and fibrinogen with cognitive decline and dementia (38,62–67). However, prospective findings have been inconsistent, particularly for investigations of CRP (68–71). Two studies found that higher CRP was associated with vascular dementia (VaD) but not AD or all-cause dementia (72,73). In our study, CRP had the weakest association with cognitive decline, suggesting that IL-6 and fibrinogen are more sensitive markers of cognitive impairment. This is not surprising as IL-6 is a major pro-inflammatory cytokine that induces the hepatic synthesis of CRP and fibrinogen (33) and fibrinogen plays a key role in both inflammation and hemostasis and vascular pathology is increasingly recognized as an important contributor to dementia (15,74–77). Fibrinogen has deleterious effects on the majority of the CNS cells and has been linked to impairments in oligodendrocyte maturation, axonal damage, and increased production of reactive oxygen species from microglia (78,79). Further, evidence shows that fibrinogen co-localizes with amyloid beta in the CNS, impairing its clearance and creating a prothrombotic and proinflammatory environment that leads to neurodegeneration (75,76).

Heterogeneity across the literature may also reflect variable age ranges, follow-up periods, and measures of cognition. In our study cohort, with a mean age of 71.9 and median follow-up of 9.3 years, inflammatory markers were not associated with dementia. However, in the Honolulu-Asia Aging Study, where a cohort with a mean age of 55 was followed for 25 years, higher CRP was associated with an increased risk of all-cause dementia and dementia subtypes (80), suggesting

that these markers may be of greater value as an early indicator of dementia in midlife. As dementia pathology progresses, inflammatory markers may begin to saturate, limiting their predictive and discriminative utility. This tracks with evidence demonstrating cerebrospinal fluid and neuroimaging biomarkers sensitive to the onset and progression of cognitive impairment in dementia subjects (81). Given dementia's long preclinical stage, such biomarkers would be of value in clinical decision-making and lengthen the therapeutic window for intervention (81).

We also found sex-specific differences of the association between fibrinogen and cognitive decline. While AF females with elevated fibrinogen had higher odds of cognitive decline, we found no significant association among AF males. In post-hoc analyses, we found that AF females were more likely to have an incident stroke than AF males (22.0% versus 18.2%, $p = 0.007$). However, when excluding those with incident stroke, the association remained significant. Given evidence for widespread sex differences in both the concentrations and effects of multiple inflammatory markers on CVD risk (82,83), future work should continue to consider sex in the evaluation of inflammatory-mediated BBB dysfunction for dementia risk.

Previous cohort studies have reported a larger association between CRP and cognitive decline among APOE Ɛ4 carriers (38,39). Contrary to our findings, a cohort study reported that higher levels of CRP were associated with an increased risk of AD only in APOE Ɛ4 carriers, particularly in the absence of cardiovascular disease (37). We found that among those with AF, APOE Ɛ4 interacted with inflammatory markers to increase the odds of cognitive decline. Whereas in the non-AF cohort, APOE Ɛ4 interacted with inflammatory markers, diminishing the odds of cognitive decline. Inflammatory-mediated BBB dysfunction may play a larger role among APOE Ɛ4 carriers with AF, who presumably have both vascular and AD pathologies contributing to cognitive decline. As the APOE Ɛ4 allele has been shown to contribute to BBB dysfunction (21),

both APOE Ɛ4- and inflammatory-mediated BBB dysfunction may exacerbate cognitive decline among those with AF. Whereas in the non-AF cohort, controlling for other vascular risk factors, these inflammatory markers may contribute less to cognitive decline and dementia among APOE Ɛ4 carriers with pure AD pathology. Domain-specific cognitive testing may provide a better understanding on how APOE carrier status influences inflammatory-related cognitive trajectories in the AF and non-AF populations. As AF is associated with decline in executive function (84), the hallmark clinical presentation of VaD (54), and APOE Ɛ4 is thought to primarily influence memory (85), AF participants with the APOE Ɛ4 allele may present faster rates of cognitive decline across multiple domains.

The strengths of this study include a large well-characterized cohort of older adults with a median follow-up period of 9.3 years. The prospective design allowed us to investigate temporal relationships between inflammatory markers and cognition, ruling out reverse causality. While previous studies are often limited by the measurement of inflammatory markers at one fixed timepoint, we were able to account for fluctuations in levels over time in primary analyses. As dementia status and onset were adjudicated by a review committee of neurologists and psychiatrists regardless of loss to follow-up or mortality, outcome misclassification is less likely. We were also able to control for many confounding demographic and vascular risk factors and account for death as a competing risk.

There were also several limitations to our study. First, due to a limited number of prevalent AF cases, we included incident AF cases in our stratified analyses. Therefore, we were not able to establish AF as a precursor to systemic inflammation. However, evidence suggests that inflammation may lead to AF and that AF in turn promotes inflammation (86), suggesting that AF may not necessarily precede inflammation. Further, younger AF patients are more likely to develop

dementia compared to older patients (87), suggestive of a dose-response relationship. As such, incident AF is unlikely to significantly contribute to dementia over the observed follow-up period. Future studies should investigate AF duration and subtype as those with the longest history or with persistent AF have the highest risk of dementia (87–91). In addition, we were unable to confirm whether those with self-reported AF truly had the condition. Therefore, subjects with self-reported AF may have been misclassified, particularly given the low anticoagulant use at baseline. Second, non-significant associations in primary analyses may be attributed to low statistical power. Third, incident stroke, TIA, and silent cerebral infarcts may be an intermediate on the causal pathway accounting for the observed associations. However, sensitivity analyses revealed that many associations remained significant when excluding those with incident stroke. Fourth, subjects with missing 3MS scores may have been more likely to be lost to follow-up due to progressive cognitive decline, biasing our results towards the null. Finally, as inflammatory markers were measured from plasma, systemic inflammation may not accurately reflect BBB dysfunction and inflammation in the CNS. Future studies should aim to use more sensitive markers of BBB dysfunction. For example, recent studies have highlighted the potential role of brain-derived extracellular vesicles (EVs) as indicators of neuropathology (92). The ability to measure brain-derived EVs indicative of central inflammation and BBB dysfunction would compliment recent studies describing EVs, and their associated cargo, that are altered in AD, Frontotemporal dementia, and small vessel disease (93–96).

Sources of Funding

This research was supported by a grant from the Canadian Institutes of Health Research (PJT 169192).

Acknowledgments

This research was supported by contracts HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, 75N92021D00006, and grants U01HL080295 and U01HL130114 from the National Heart, Lung, and Blood Institute (NHLBI), with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided by R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. Int J Stroke. 2020;1747493019897870–1747493019897870.
2. Canadian Institute for Health Information. Dementia in Canada: Summary [Internet]. [cited 2021 Jan 30]. Available from: https://www.cihi.ca/en/dementia-in-canada/dementia-in-canada-summary
3. Wolf PA, Abbott RD, Kannel WB. Atrial Fibrillation as an Independent Risk Factor for Stroke: The Framingham Study. Stroke. 1991 Aug;22(8):983–8.
4. Kuźma E, Lourida I, Moore SF, Levine DA, Ukoumunne OC, Llewellyn DJ. Stroke and dementia risk: A systematic review and meta-analysis. Alzheimers Dement J Alzheimers Assoc. 2018 Nov;14(11):1416–26.

5. Kalantarian S, Stern TA, Mansour M, Ruskin JN. Cognitive Impairment Associated With Atrial Fibrillation. Ann Intern Med. 2013 Mar 5;158(5_Part_1):338–46.

6. Madhavan M, Graff-Radford J, Piccini JP, Gersh BJ. Cognitive dysfunction in atrial fibrillation. Nat Rev Cardiol. 2018 Dec;15(12):744–56.

7. Crandall MA, Horne BD, Day JD, Anderson JL, Muhlestein JB, Crandall BG, et al. Atrial Fibrillation and CHADS2 Risk Factors are Associated with Highly Sensitive C-Reactive Protein Incrementally and Independently. Pacing Clin Electrophysiol. 2009;32(5):648–52.

8. Phillips KP. Role of Inflammation in Initiation and Perpetuation of Atrial Fibrillation: A Systematic Review of the Published Data. J Atr Fibrillation. 2013 Oct 31;6(3):935.

9. Anderson JL, Allen Maycock CA, Lappé DL, Crandall BG, Horne BD, Bair TL, et al. Frequency of elevation of C-reactive protein in atrial fibrillation. Am J Cardiol. 2004 Nov 15;94(10):1255–9.

10. Guo Y, Lip GYH, Apostolakis S. Inflammation in Atrial Fibrillation. J Am Coll Cardiol. 2012 Dec 4;60(22):2263–70.

11. Mukamal KJ, Tolstrup JS, Friberg J, Grønbaek M, Jensen G. Fibrinogen and Albumin Levels and Risk of Atrial Fibrillation in Men and Women (the Copenhagen City Heart Study). Am J Cardiol. 2006 Jul 1;98(1):75–81.

12. Holmes C. Review: systemic inflammation and Alzheimer's disease. Neuropathol Appl Neurobiol. 2013 Feb;39(1):51–68.

13. McGeer PL, McGeer EG. Local neuroinflammation and the progression of Alzheimer's disease. J Neurovirol. 2002 Dec;8(6):529–38.

14. McGeer PL, Rogers J, McGeer EG. Inflammation, Antiinflammatory Agents, and Alzheimer's Disease: The Last 22 Years. J Alzheimers Dis JAD. 2016 Oct 4;54(3):853–7.

15. Snyder HM, Corriveau RA, Craft S, Faber JE, Greenberg SM, Knopman D, et al. Vascular contributions to cognitive impairment and dementia including Alzheimer's disease. Alzheimers Dement. 2015 Jun 1;11(6):710–7.

16. Hussain B, Fang C, Chang J. Blood–Brain Barrier Breakdown: An Emerging Biomarker of Cognitive Impairment in Normal Aging and Dementia. Front Neurosci. 2021;15:978.

17. Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018 Mar;14(3):133–50.

18. Montagne A, Barnes SR, Sweeney MD, Halliday MR, Sagare AP, Zhao Z, et al. Blood-Brain Barrier Breakdown in the Aging Human Hippocampus. Neuron. 2015 Jan 21;85(2):296–302.

19. Cai Z, Qiao PF, Wan CQ, Cai M, Zhou NK, Li Q. Role of Blood-Brain Barrier in Alzheimer's Disease. J Alzheimers Dis. 2018 Jan 1;63(4):1223–34.

20. Zenaro E, Piacentino G, Constantin G. The blood-brain barrier in Alzheimer's disease. Neurobiol Dis. 2017 Nov;107:41–56.

21. Montagne A, Nation DA, Sagare AP, Barisano G, Sweeney MD, Chakhoyan A, et al. APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. Nature. 2020 May;581(7806):71–6.

22. Nation DA, Sweeney MD, Montagne A, Sagare AP, D'Orazio LM, Pachicano M, et al. Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. Nat Med. 2019 Feb;25(2):270–6.

23. Galenko O, Jacobs V, Knight S, Bride D, Cutler MJ, Muhlestein JB, et al. Circulating Levels of Biomarkers of Cerebral Injury in Patients with Atrial Fibrillation. Am J Cardiol. 2019 Dec 1;124(11):1697–700.

24. Marcus GM, Whooley MA, Glidden DV, Pawlikowska L, Zaroff JG, Olgin JE. Interleukin-6 and atrial fibrillation in patients with coronary artery disease: Data from the Heart and Soul Study. Am Heart J. 2008 Feb 1;155(2):303–9.

25. Ding WY, Gupta D, Lip GYH. Atrial fibrillation and the prothrombotic state: revisiting Virchow's triad in 2020. Heart. 2020 Oct 1;106(19):1463–8.

26. Hsuchou H, Kastin AJ, Mishra PK, Pan W. C-reactive protein increases BBB permeability: implications for obesity and neuroinflammation. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol. 2012;30(5):1109–19.

27. Rochfort KD, Collins LE, Murphy RP, Cummins PM. Downregulation of blood-brain barrier phenotype by proinflammatory cytokines involves NADPH oxidase-dependent ROS generation: consequences for interendothelial adherens and tight junctions. PloS One. 2014;9(7):e101815.

28. Takeshita Y, Fujikawa S, Serizawa K, Fujisawa M, Matsuo K, Nemoto J, et al. New BBB Model Reveals That IL-6 Blockade Suppressed the BBB Disorder, Preventing Onset of NMOSD. Neurol – Neuroimmunol Neuroinflammation [Internet]. 2021 Nov 1 [cited 2022 Mar 26];8(6). Available from: https://nn.neurology.org/content/8/6/e1076

29. Paul J, Strickland S, Melchor JP. Fibrin deposition accelerates neurovascular damage and neuroinflammation in mouse models of Alzheimer's disease. J Exp Med [Internet]. 2007 [cited 2022 Mar 26];204(8). Available from: https://www.readcube.com/articles/10.1084%2Fjem.20070304

30. Ryu JK, McLarnon JG. A leaky blood–brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. J Cell Mol Med. 2009;13(9a):2911–25.

31. PATIBANDLA PK, TYAGI N, DEAN WL, TYAGI SC, ROBERTS AM, LOMINADZE D. Fibrinogen Induces Alterations of Endothelial Cell Tight Junction Proteins. J Cell Physiol. 2009 Oct;221(1):195–203.

32. Elwood E, Lim Z, Naveed H, Galea I. The effect of systemic inflammation on human brain barrier function. Brain Behav Immun. 2017 May;62:35–40.

33. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett. 1989 Jan 2;242(2):237–9.

34. Ansar W, Ghosh S. Clinical significance of C-reactive protein. Singapore: Springer; 2020.

35. Su JH, Luo MY, Liang N, Gong SX, Chen W, Huang WQ, et al. Interleukin-6: A Novel Target for Cardio-Cerebrovascular Diseases. Front Pharmacol [Internet]. 2021 [cited 2022 Apr 8];12. Available from: https://www.frontiersin.org/article/10.3389/fphar.2021.745061

36. Stec JJ, Silbershatz H, Tofler GH, Matheney TH, Sutherland P, Lipinska I, et al. Association of Fibrinogen With Cardiovascular Risk Factors and Cardiovascular Disease in the Framingham Offspring Population. Circulation. 2000 Oct 3;102(14):1634–8.

37. Tao Q, Ang TFA, DeCarli C, Auerbach SH, Devine S, Stein TD, et al. Association of Chronic Low-grade Inflammation With Risk of Alzheimer Disease in ApoE4 Carriers. JAMA Netw Open. 2018 Oct 19;1(6):e183597.

38. Noble JM, Manly JJ, Schupf N, Tang MX, Mayeux R, Luchsinger JA. Association of C-Reactive Protein With Cognitive Impairment. Arch Neurol. 2010 Jan 1;67(1):87–92.

39. Lima TAS, Adler AL, Minett T, Matthews FE, Brayne C, Marioni RE, et al. C-reactive protein, APOE genotype and longitudinal cognitive change in an older population. Age Ageing. 2014 Mar 1;43(2):289–92.

40. Duarte-Guterman P, Albert AY, Inkster AM, Barha CK, Galea LAM, Alzheimer's Disease Neuroimaging Initiative. Inflammation in Alzheimer's Disease: Do Sex and APOE Matter? J Alzheimers Dis JAD. 2020;78(2):627–41.

41. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, et al. The cardiovascular health study: Design and rationale. Ann Epidemiol. 1991 Feb 1;1(3):263–76.

42. Fitzpatrick AL, Kuller LH, Ives DG, Lopez OL, Jagust W, Breitner JCS, et al. Incidence and Prevalence of Dementia in the Cardiovascular Health Study. J Am Geriatr Soc. 2004;52(2):195–204.

43. Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, et al. Relationship of C-Reactive Protein to Risk of Cardiovascular Disease in the Elderly. Arterioscler Thromb Vasc Biol. 1997 Jun 1;17(6):1121–7.

44. Cushman M, Cornell ES, Howard PR, Bovill EG, Tracy RP. Laboratory methods and quality assurance in the Cardiovascular Health Study. Clin Chem. 1995 Feb 1;41(2):264–70.

45. Lopez OL, Kuller LH, Fitzpatrick A, Ives D, Becker JT, Beauchamp N. Evaluation of dementia in the cardiovascular health cognition study. Neuroepidemiology. 2003 Feb;22(1):1–12.

46. Teng EL, Chui HC. The Modified Mini-Mental State (3MS) examination. J Clin Psychiatry. 1987 Aug;48(8):314–8.

47. Bryan RN, Manolio TA, Schertz LD, Jungreis C, Poirier VC, Elster AD, et al. A method for using MR to evaluate the effects of cardiovascular disease on the brain: the cardiovascular health study. Am J Neuroradiol. 1994 Oct 1;15(9):1625–33.

48. Kowey PR, Naccarelli GV. Atrial fibrillation. New York: Marcel Dekker; 2005. xvii+365. (Fundamental and clinical cardiology ; v. 49).

49. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. JAMA. 1994 Mar 16;271(11):840–4.

50. Psaty BM, Manolio TA, Kuller LH, Kronmal RA, Cushman M, Fried LP, et al. Incidence of and risk factors for atrial fibrillation in older adults. Circulation. 1997 Oct 7;96(7):2455–61.

51. Friberg L, Rosenqvist M. Less dementia with oral anticoagulation in atrial fibrillation. Eur Heart J. 2018 Feb 7;39(6):453–60.

52. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. The Lancet. 2020 Aug 8;396(10248):413–46.

53. Park CM, Williams ED, Chaturvedi N, Tillin T, Stewart RJ, Richards M, et al. Associations Between Left Ventricular Dysfunction and Brain Structure and Function: Findings From the SABRE (Southall and Brent Revisited) Study. J Am Heart Assoc. 2017;6(4).

54. Joseph F. Quinn. Dementia. John Wiley & Sons, Ltd; 2014.

55. Qiu C, Winblad B, Marengoni A, Klarin I, Fastbom J, Fratiglioni L. Heart Failure and Risk of Dementia and Alzheimer Disease: A Population-Based Cohort Study. Arch Intern Med. 2006 May 8;166(9):1003–8.

56. Psaty BM, Kuller LH, Bild D, Burke GL, Kittner SJ, Mittelmark M, et al. Methods of assessing prevalent cardiovascular disease in the Cardiovascular Health Study. Ann Epidemiol. 1995 Jul 1;5(4):270–7.

57. Kuller LH, Shemanski L, Manolio T, Haan M, Fried L, Bryan N, et al. Relationship Between ApoE, MRI Findings, and Cognitive Function in the Cardiovascular Health Study. Stroke. 1998 Feb;29(2):388–98.

58. Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. J Lipid Res. 1990 Mar;31(3):545–8.

59. Lee, Katherine J, Tilling, Kate M, Cornish, Rosie P, Little, Roderick J A, Bell, Melanie L, Goetghebeur, Els, et al. Framework for the treatment and reporting of missing data in observational studies: The Treatment And Reporting of Missing data in Observational Studies framework. J Clin Epidemiol. 2021 Jun 1;134:79–88.

60. Austin PC, Lee DS, Fine JP. Introduction to the Analysis of Survival Data in the Presence of Competing Risks. Circulation. 2016 Feb 9;133(6):601–9.

61. Wardenaar K. Latent Class Growth Analysis and Growth Mixture Modeling using R: A tutorial for two R-packages and a comparison with Mplus. [Internet]. PsyArXiv; 2020 [cited 2022 Jan 16]. Available from: https://psyarxiv.com/m58wx/

62. Kravitz BA, Corrada MM, Kawas CH. Elevated C-reactive protein levels are associated with prevalent dementia in the oldest-old. Alzheimers Dement J Alzheimers Assoc. 2009 Jul;5(4):318–23.

63. Ravaglia G, Forti P, Maioli F, Brunetti N, Martelli M, Servadei L, et al. Serum C-Reactive Protein and Cognitive Function in Healthy Elderly Italian Community Dwellers. J Gerontol Ser A. 2005 Aug 1;60(8):1017–21.

64. Watanabe Y, Kitamura K, Nakamura K, Sanpei K, Wakasugi M, Yokoseki A, et al. Elevated C-Reactive Protein Is Associated with Cognitive Decline in Outpatients of a

General Hospital: The Project in Sado for Total Health (PROST). Dement Geriatr Cogn Disord EXTRA. 2016 Jan 19;6(1):10–9.

65. Wersching H, Duning T, Lohmann H, Mohammadi S, Stehling C, Fobker M, et al. Serum C-reactive protein is linked to cerebral microstructural integrity and cognitive function. Neurology. 2010 Mar 30;74(13):1022–9.

66. Roberts RO, Geda YE, Knopman DS, Christianson TJH, Pankratz VS, Kullo IJ, et al. Association of C-reactive protein with mild cognitive impairment. Alzheimers Dement J Alzheimers Assoc. 2009 Sep;5(5):398–405.

67. Stott DJ, Spilg E, Campbell AM, Rumley A, Mansoor MA, Lowe GD. Haemostasis in ischaemic stroke and vascular dementia. Blood Coagul Fibrinolysis Int J Haemost Thromb. 2001 Dec;12(8):651–7.

68. Dik MG, Jonker C, Hack CE, Smit JH, Comijs HC, Eikelenboom P. Serum inflammatory proteins and cognitive decline in older persons. Neurology. 2005 Apr 26;64(8):1371–7.

69. Singh-Manoux A, Dugravot A, Brunner E, Kumari M, Shipley M, Elbaz A, et al. Interleukin-6 and C-reactive protein as predictors of cognitive decline in late midlife. Neurology. 2014 Aug 5;83(6):486–93.

70. Rafnsson SB, Deary IJ, Smith FB, Whiteman MC, Rumley A, Lowe GDO, et al. Cognitive Decline and Markers of Inflammation and Hemostasis: The Edinburgh Artery Study. J Am Geriatr Soc. 2007;55(5):700–7.

71. Yaffe K, Lindquist K, Penninx BW, Simonsick EM, Pahor M, Kritchevsky S, et al. Inflammatory markers and cognition in well-functioning African-American and white elders. Neurology. 2003 Jul 8;61(1):76–80.

72. Hsu PF, Pan WH, Yip BS, Chen RCY, Cheng HM, Chuang SY. C-Reactive Protein Predicts Incidence of Dementia in an Elderly Asian Community Cohort. J Am Med Dir Assoc. 2017 Mar 1;18(3):277.e7-277.e11.

73. Engelhart MJ, Geerlings MI, Meijer J, Kiliaan A, Ruitenberg A, van Swieten JC, et al. Inflammatory Proteins in Plasma and the Risk of Dementia: The Rotterdam Study. Arch Neurol. 2004 May 1;61(5):668–72.

74. James BD, Bennett DA, Boyle PA, Leurgans S, Schneider JA. Dementia From Alzheimer Disease and Mixed Pathologies in the Oldest Old. JAMA. 2012 May 2;307(17):1798–800.

75. Cortes-Canteli M, Paul J, Norris EH, Bronstein R, Ahn HJ, Zamolodchikov D, et al. Fibrinogen and β-amyloid association alters thrombosis and fibrinolysis: a possible contributing factor to Alzheimer's disease. Neuron. 2010 Jun 10;66(5):695–709.

76. Cortes-Canteli M, Zamolodchikov D, Ahn HJ, Strickland S, Norris EH. Fibrinogen and Altered Hemostasis in Alzheimer's Disease. de la Torre J, editor. J Alzheimers Dis. 2012 Oct 29;32(3):599–608.

77. Schneider, Julie A., Arvanitakis, Zoe, Bang, Woojeong, Bennett, David A. Mixed brain pathologies account for most dementia cases in community-dwelling older persons SYMBOL. Neurology. 69(24):2197–204.

78. Davalos D, Kyu Ryu J, Merlini M, Baeten KM, Le Moan N, Petersen MA, et al. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. Nat Commun. 2012 Nov 27;3(1):1227.

79. Petersen MA, Ryu JK, Chang KJ, Etxeberria A, Bardehle S, Mendiola AS, et al. Fibrinogen Activates BMP Signaling in Oligodendrocyte Progenitor Cells and Inhibits Remyelination after Vascular Damage. Neuron. 2017 Dec 6;96(5):1003-1012.e7.

80. Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ. Early inflammation and dementia: A 25-year follow-up of the Honolulu-Asia aging study. Ann Neurol. 2002;52(2):168–74.

81. Counts SE, Ikonomovic MD, Mercado N, Vega IE, Mufson EJ. Biomarkers for the Early Detection and Progression of Alzheimer's Disease. Neurotherapeutics. 2017 Jan 1;14(1):35–53.

82. Lau ES, Paniagua SM, Guseh JS, Bhambhani V, Zanni MV, Courchesne P, et al. Sex Differences in Circulating Biomarkers of Cardiovascular Disease. J Am Coll Cardiol. 2019 Sep 24;74(12):1543–53.

83. Li T, Wang F, Peng R, Pei S, Hou Z, Lu B, et al. Sex-related differences in the association between plasma fibrinogen and non-calcified or mixed coronary atherosclerotic plaques. Biol Sex Differ. 2018 Dec 5;9(1):51.

84. Nishtala A, Piers RJ, Himali JJ, Beiser AS, Davis-Plourde KL, Saczynski JS, et al. Atrial fibrillation and cognitive decline in the Framingham Heart Study. Heart Rhythm. 2018 Feb 1;15(2):166–72.

85. Caselli RJ, Dueck AC, Osborne D, Sabbagh MN, Connor DJ, Ahern GL, et al. Longitudinal Modeling of Age-Related Memory Decline and the APOE ε4 Effect. N Engl J Med. 2009 Jul 16;361(3):255–63.

86. Korantzopoulos P, Letsas KP, Tse G, Fragakis N, Goudis CA, Liu T. Inflammation and atrial fibrillation: A comprehensive review. J Arrhythmia. 2018 Jun 4;34(4):394–401.

87. de Bruijn RFAG, Heeringa J, Wolters FJ, Franco OH, Stricker BHC, Hofman A, et al. Association Between Atrial Fibrillation and Dementia in the General Population. JAMA Neurol. 2015;72(11):1–7.

88. Bunch TJ, Weiss JP, Crandall BG, May HT, Bair TL, Osborn JS, et al. Atrial fibrillation is independently associated with senile, vascular, and Alzheimer's dementia. Heart Rhythm. 2010 Apr 1;7(4):433–7.

89. OTT A, BRETELER MMB, DE BRUYNE MC, VAN HARSKAMP F, GROBBEE DE, HOFMAN A. Atrial fibrillation and dementia in a population-based study: The Rotterdam study. Stroke 1970. 1997;28(2):316–21.

90. Kim D, Yang PS, Joung B. Prevention of Dementia in Patients with Atrial Fibrillation. Korean Circ J. 2021 Mar 11;51(4):308–19.

91. Stefansdottir H, Arnar DO, Aspelund T, Sigurdsson S, Jonsdottir MK, Hjaltason H, et al. Atrial fibrillation is associated with reduced brain volume and cognitive function independent of cerebral infarcts. Stroke. 2013 Apr;44(4):1020–5.

92. Badhwar A, Haqqani AS. Biomarker potential of brain-secreted extracellular vesicles in blood in Alzheimer's disease. Alzheimers Dement Diagn Assess Dis Monit. 2020;12(1):e12001.

93. Elahi FM, Harvey D, Altendahl M, Brathaban N, Fernandes N, Casaletto KB, et al. Elevated complement mediator levels in endothelial-derived plasma exosomes implicate endothelial innate inflammation in diminished brain function of aging humans. Sci Rep. 2021 Aug 10;11(1):16198.

94. Winston CN, Goetzl EJ, Akers JC, Carter BS, Rockenstein EM, Galasko D, et al. Prediction of conversion from mild cognitive impairment to dementia with neuronally derived blood exosome protein profile. Alzheimers Dement Diagn Assess Dis Monit. 2016;3(1):63–72.

95. Kapogiannis D, Mustapic M, Shardell MD, Berkowitz ST, Diehl TC, Spangler RD, et al. Association of Extracellular Vesicle Biomarkers With Alzheimer Disease in the Baltimore Longitudinal Study of Aging. JAMA Neurol. 2019 Nov 1 ;76(11) :1340–51.

96. Longobardi A, Benussi L, Nicsanu R, Bellini S, Ferrari C, Saraceno C, et al. Plasma Extracellular Vesicle Size and Concentration Are Altered in Alzheimer's Disease, Dementia With Lewy Bodies, and Frontotemporal Dementia. Front Cell Dev Biol [Internet]. 2021 [cited 2022 Apr 7];9. Available from: https://www.frontiersin.org/article/10.3389/fcell.2021.667369

Supplemental Materials

Table 5-5. Cause-specific Cox proportional hazards model showing crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) between inflammatory markers and incident dementia or death, excluding those with MCI (n = 577)

Cohort	Time-varying exposure†	Dementia HR (95% CI)		Death HR (95% CI)	
		Crude	Adjusted*	Crude	Adjusted*
Adults with AF prior to incident dementia (n=409)	CRP	1.04 (0.89-1.21)	0.98 (0.83-1.15)	1.04 (0.97-1.12)	1.05 (0.97-1.13)
	IL-6	1.16 (0.88-1.54)	1.00 (0.72-1.38)	1.17 (1.02-1.33)	1.09 (0.94-1.25)
	Fibrinogen	1.06 (0.73-1.54)	0.90 (0.60-1.35)	1.05 (0.90-1.23)	1.04 (0.87-1.24)
Adults with no AF (n=2,389)	CRP	0.97 (0.91-1.03)	0.97 (0.90-1.04)	1.02 (0.99-1.05)	1.03 (0.99-1.06)
	IL-6	1.27 (1.14-1.42)	1.10 (0.98-1.25)	1.23 (1.16-1.30)	1.18 (1.11-1.26)
	IL-6*APOE Ɛ4	1.08 (0.88-1.34)	0.88 (0.70-1.12)		
	Fibrinogen	1.25 (1.08-1.44)	1.09 (0.93-1.28)	1.13 (1.05-1.22)	1.08 (1.00-1.18)
Overall cohort (n=2,798)	CRP	0.98 (0.92-1.03)	0.97 (0.91-1.03)	1.03 (1.00-1.06)	1.03 (1.00-1.06)
	IL-6	1.25 (1.13-1.39)	1.08 (0.97-1.21)	1.23 (1.17-1.30)	1.17 (1.11-1.24)
	IL-6*APOE Ɛ4	1.25 (1.11-1.40)	0.88 (0.71-1.10)		
	Fibrinogen	1.22 (1.07-1.40)	1.07 (0.92-1.24)	1.13 (1.05-1.21)	1.09 (1.02-1.18)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, incident stroke (time-varying), left ventricular hypertrophy, congestive hart failure, myocardial infarction, antihypertensives, lipid-lowering medication, and anticoagulant use (time-varying).

†CRP and IL-6 were log_2 transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Table 5-6. Cause-specific Cox proportional hazards model showing crude and adjusted hazard ratios (HR) and 95% confidence intervals (95% CI) between inflammatory markers and incident dementia or death, excluding those with incident stroke (n = 671)

Cohort	Time-varying exposure†	Dementia HR (95% CI)		Death HR (95% CI)	
		Crude	Adjusted*	Crude	Adjusted*
Adults with AF prior to incident dementia (n=356)	CRP	0.96 (0.76-1.20)	0.95 (0.73-1.24)	1.02 (0.95-1.09)	1.01 (0.92-1.10)
	IL-6	1.00 (0.66-1.51)	0.83 (0.53-1.32)	1.16 (1.01-1.33)	1.08 (0.93-1.26)
	Fibrinogen	0.84 (0.49-1.45)	0.70 (0.36-1.36)	1.04 (0.90-1.22)	1.02 (0.86-1.21)
Adults with no AF (n=2,254)	CRP	0.93 (0.86-1.00)	0.95 (0.87-1.03)	1.03 (0.99-1.06)	1.03 (1.00-1.07)
	IL-6	1.24 (1.09-1.42)	1.11 (0.96-1.29)	1.22 (1.15-1.29)	1.18 (1.11-1.25)
	IL-6*APOE Ɛ4	1.01 (0.78-1.29)	0.91 (0.69-1.21)		
	Fibrinogen	1.25 (1.05-1.48)	1.11 (0.92-1.34)	1.14 (1.06-1.23)	1.08 (1.00-1.17)
Overall cohort (n=2,610)	CRP	0.93 (0.87-1.00)	0.95 (0.88-1.03)	1.03 (1.00-1.06)	1.03 (1.00-1.06)
	IL-6	1.20 (1.05-1.36)	1.07 (0.93-1.23)	1.22 (1.16-1.29)	1.16 (1.10-1.23)
	IL-6*APOE Ɛ4	0.97 (0.76-1.23)	0.86 (0.66-1.13)		
	Fibrinogen	1.19 (1.01-1.40)	1.09 (0.91-1.30)	1.13 (1.06-1.21)	1.08 (1.00-1.16)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive hart failure, myocardial infarction, antihypertensives, lipid-lowering medication, and anticoagulant use (time-varying).

†CRP and IL-6 were log_2 transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Table 5-7. Binomial logistic regression model showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between inflammatory markers and cognitive relative to class 1 (Stable), excluding those with MCI (n = 577)

Cohort	Baseline Exposure†	Class 2: Cognitive Decline OR (95% CI)	
		Crude	**Adjusted***
Adults with prevalent or incident AF (n=988)	CRP	1.09 (1.04-1.14)	1.03 (0.98-1.08)
	CRP*APOE Ɛ4	1.39 (1.27-1.54)	1.24 (1.11-1.38)
	IL-6	1.34 (1.25-1.45)	1.27 (1.16-1.40)
Adults without AF (n=1,810)	IL-6	1.36 (1.29-1.44)	1.20 (1.12-1.28)
	IL-6*APOE Ɛ4	1.19 (1.06-1.35)	1.04 (0.91-0.1.16)
	Fibrinogen	1.11 (1.03-1.20)	0.93 (0.85-1.02)
	Fibrinogen*APOE Ɛ4	0.93 (0.79-1.09)	0.77 (0.65-0.92)
Overall cohort (n=2,798)	CRP	1.06 (1.03-1.09)	1.00 (0.97-1.04)
	IL-6	1.35 (1.29-1.42)	1.21 (1.15-1.28)
	IL-6*APOE Ɛ4	1.32 (1.20-1.46)	1.13 (1.02-1.27)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, and baseline use of antihypertensives, lipid-lowering medications and anticoagulants.

†CRP and IL-6 were $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Table 5-8. Sex-specific binomial logistic regression models showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between inflammatory markers and cognitive relative to class 1 (Stable), excluding those with MCI (n = 341 females; n = 236 males)

Cohort	Baseline Exposure†	Class 2: Cognitive Decline OR (95% CI)			
		Female-specific model		Male-specific model	
		Crude	Adjusted*	Crude	Adjusted*
Adults with prevalent or incident AF (n=1,174)	Fibrinogen	1.75 (1.51-2.01)	1.57 (1.32-1.86)	1.09 (0.94-1.25)	0.93 (0.80-1.08)
	Fibrinogen*APOE Ɛ4	3.44 (2.53-4.67)	3.42 (2.40-4.88)		
Adults without AF (n=1,810)	CRP	1.05 (1.01-1.10)	1.03 (0.97-1.08)	1.03 (0.97-1.09)	0.94 (0.88-1.00)
Overall cohort (n=2,798)	Fibrinogen	1.35 (1.25-1.46)	1.14 (1.04-1.25)	1.03 (0.94-1.13)	0.91 (0.82-1.00)
	Fibrinogen*APOE Ɛ4			0.93 (0.76-1.12)	0.71 (0.57-0.87)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, and baseline use of antihypertensives, lipid-lowering medications and anticoagulants.

†CRP was $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Table 5-9. Binomial logistic regression model showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between inflammatory markers and cognitive relative to class 1 (Stable), excluding those with incident stroke (n = 671)

Cohort	Baseline Exposure†	Class 2: Cognitive Decline OR (95% CI)	
		Crude	Adjusted*
Adults with prevalent or incident AF (n=836)	CRP	1.06 (1.01-1.11)	1.05 (0.99-1.10)
	CRP*APOE Ɛ4	1.20 (1.07-1.34)	1.16 (1.02-1.33)
	IL-6	1.24 (1.14-1.34)	1.21 (1.10-1.33)
Adults without AF (n=1,774)	IL-6	1.34 (1.26-1.41)	1.16 (1.09-1.24)
	IL-6*APOE Ɛ4	1.07 (0.95-1.20)	0.84 (0.73-0.96)
	Fibrinogen	1.13 (1.05-1.21)	0.95 (0.87-1.03)
	Fibrinogen*APOE Ɛ4	0.97 (0.83-1.14)	0.76 (0.64-0.91)
Overall cohort (n=2,610)	CRP	1.08 (1.05-1.10)	1.03 (1.00-1.07)
	IL-6	1.30 (1.24-1.36)	1.18 (1.12-1.24)
	IL-6*APOE Ɛ4	1.14 (1.03-1.26)	0.92 (0.82-1.03)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, and baseline use of antihypertensives, lipid-lowering medications and anticoagulants.

†CRP and IL-6 were $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Table 5-10. Sex-specific binomial logistic regression models showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between inflammatory markers and cognitive relative to class 1 (Stable), excluding those with incident stroke (n = 431 females; n = 240 males)

Cohort	Baseline Exposure†	Class 2: Cognitive Decline OR (95% CI)			
		Female-specific model		Male-specific model	
		Crude	Adjusted*	Crude	Adjusted*
Adults with prevalent or incident AF (n=1,174)	Fibrinogen	1.49 (1.29-1.73)	1.41 (1.18-1.68)	1.23 (1.06-1.43)	1.06 (0.89-1.26)
	Fibrinogen*APOE Ɛ4	2.18 (1.59-2.99)	1.80 (1.26-2.55)		
Adults without AF (n=1,774)	CRP	1.11 (1.06-1.15)	1.07 (1.02-1.14)	1.07 (1.02-1.13)	0.98 (0.92-1.04)
Overall cohort (n=2,610)	Fibrinogen	1.35 (1.25-1.46)	1.14 (1.04-1.26)	1.03 (0.94-1.13)	0.93 (0.83-1.03)
	Fibrinogen*APOE Ɛ4			0.93 (0.77-1.12)	0.75 (0.60-0.95)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, and baseline use of antihypertensives, lipid-lowering medications and anticoagulants.

†CRP was $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Exploratory Analyses: Worsening WMG on MRI

Worsening WMG was measured on MRI scans taken approximately 5 years apart (130). Details of MRI methods have been previously published (136). Briefly, MRI scans were performed using standardized sagittal and axial T1-weighted, spin density-, and T2- weighted images (136). Axial images were 5 mm thick without interslice gaps (136). MRI scans were independently evaluated by two trained neuroradiologists, blinded to participant information, for the presence of cerebral infarcts (136). Infarcts were defined as lesions without mass effect but with abnormal signal

intensity > 3 mm in size in a vascular distribution (136). Based on a library of template scans, they were graded using a 10-point scale, ranging from 0 (no lesions) to 9 (most lesions) (136). Change in WMG was categorized into 3 levels: no change or decrease in grade, 1 grade increase, or 2 or more-grade increase.

Table 5-11. Multinomial logistic regression model showing crude and adjusted odds ratios (OR) and 95% confidence intervals (95% CI) between inflammatory markers and worsening white matter grade between MRI 1 and MRI 2 (ref: no change or improved WMG, n = 1,050)

Cohort	Baseline Exposure†	Worsening White Matter Grade OR (95% CI)			
		1 Grade (n = 659)		≥ 2 Grades (n = 351)	
		Crude	Adjusted*	Crude	Adjusted*
Adults with prevalent or incident AF (n=736)	CRP	0.99 (0.88-1.11)	1.00 (0.87-1.14)	0.97 (0.83-1.12)	0.98 (0.83-1.16)
	IL-6	1.01 (0.83-1.24)	1.05 (0.85-1.31)	0.90 (0.69-1.16)	0.85 (0.64-1.14)
	Fibrinogen	1.00 (0.77-1.31)	0.95 (0.71-1.27)	0.91 (0.65-1.28)	0.85 (0.59-1.23)
Adults without AF (n=1,324)	CRP	1.03 (0.95-1.13)	1.02 (0.92-1.12)	1.06 (0.95-1.18)	1.08 (0.96-1.21)
	IL-6	1.29 (1.11-1.51)	1.29 (1.09-1.54)	1.15 (0.95-1.39)	1.14 (0.92-1.41)
	Fibrinogen	1.03 (0.84-1.26)	1.02 (0.82-1.27)	1.08 (0.84-1.38)	1.09 (0.84-1.40)
Overall cohort (n=2,060)	CRP	1.02 (0.95-1.09)	1.01 (0.93-1.09)	1.02 (0.94-1.12)	1.05 (0.95-1.15)
	IL-6	1.18 (1.05-1.34)	1.18 (1.03-1.35)	1.05 (0.90-1.22)	1.03 (0.87-1.22)
	Fibrinogen	1.02 (0.86-1.19)	0.99 (0.84-1.18)	1.01 (0.83-1.24)	1.01 (0.82-1.25)

*Adjusting for age, sex, race, education, smoking, hypertension, BMI, cholesterol, diabetes, baseline stroke, left ventricular hypertrophy, congestive heart failure, myocardial infarction, and baseline use of antihypertensives, lipid-lowering medications and anticoagulants.

†CRP and IL-6 were $\log_2$ transformed to correct for skewness and fibrinogen was scaled down by a factor of 100.

Chapter 6. Discussion

6.1 Interpretation of Findings

There is a critical need to better understand the pathophysiological pathways linking AF to dementia. Current models of AF-dementia pathophysiology do not consider the potential role of the BBB and few studies have examined the contributions of BBB dysfunction to this relationship. Further, as measuring BBB dysfunction is difficult in a clinical setting, human studies are scarce, particularly in an AF population (13). Using circulating inflammatory markers known to influence BBB integrity, we provided greater insight into the contribution of inflammatory-mediated BBB dysfunction to the risk of cognitive decline and dementia in two cardiovascular cohorts. Among those with AF, elevated levels of CRP, IL-6, and fibrinogen were associated with cognitive decline but not dementia, providing preliminary evidence for inflammatory-mediated BBB dysfunction as a potential contributing pathway linking AF to cognitive decline.

The associations with cognitive decline and not dementia observed in the present analyses may be an indication that inflammatory-mediated BBB dysfunction could play an early role in dementia pathology. Specifically, it is becoming increasingly apparent that biomarkers of dementia have unique trajectories relative to pathophysiological and clinical disease progression (137). For instance, low cerebrospinal fluid (CSF) Aβ, a biomarker of AD, has been shown to reach a minimum concentration 5 to 10 years before progression from MCI to AD (138). Further, findings from other clinical studies support a prodromal increase in Aβ deposition in the brain that gradually reaches a maximum threshold upon clinical presentation of MCI (139–141). Another study found brain inflammation in patients with MCI, providing further evidence that these biomarkers may be an indicator of early clinical onset (55,142). Moreover, inflammation may be correlated with Aβ deposition. Several previous studies have reported increased inflammation in Aβ positive MCI

(143) and dementia cases (9,51). Interestingly, another study reported that cortical inflammation was positively correlated with increased Aβ load in MCI cases, however inflammation declined when Aβ load approached levels typically seen in individuals with a clinical diagnosis of dementia (144). *Jack et al. (2010)* proposed that biomarkers of dementia may follow a non-linear sigmoid shaped trajectory where the occurrence of a maximum threshold varies over the course of dementia progression (137). Consistent with these data, we showed associations between inflammatory markers during the preclinical stage of dementia (i.e., cognitive decline) and no associations thereafter, suggesting that these markers may reach a saturation point prior to the onset of dementia. Future research with extended follow-up of preclinical individuals with AF and repeated biomarker measurement is needed to determine how biomarker trajectories correlate with early disease pathology and stages of dementia progression.

The negative findings for dementia in the AF sub-cohorts may also be due to limitations in our study design. In the REGARDS study, we selected an optimal cut-off score from the SIS to ascertain dementia cases. In addition, considering the limited and conflicting data on the validity of this tool for dementia diagnosis (127,145), outcome misclassification is possible. Using data from the CHS, the second manuscript addressed these important limitations as a committee of neurologists and psychiatrists classified dementia status based on neuropsychological evaluations, MRI scans, and other data collected over the course of follow-up. However, our analyses were not sufficiently powered in the AF sub-cohort. Further, our stratified analyses included both prevalent and incident AF cases. Consequently, AF did not necessarily precede inflammation and a shorter amount of time accrued between incident AF and dementia diagnosis. As highlighted in *Chapter 2.3*, younger patients with the longest history of AF seem to have the highest risk of dementia (60,61,67,146). As such, incident AF may be unlikely to be a significant contributor to dementia

over a short interval period. Future studies should prioritize inclusion of a sufficient number of ECG-confirmed prevalent AF cases to be properly powered to establish a temporal association with cognitive outcomes. A younger cohort of AF cases at baseline with a longer follow-up period may further elucidate this association. Moreover, as evidence suggests that the risk of dementia is higher among those with persistent rather than paroxysmal AF (84), future studies may consider investigating associations by AF subtype.

We also showed that both sex and APOE genotype were important effect modifiers of the observed associations. In the CHS cohort, we found that sex significantly modified the association between fibrinogen and cognitive decline. Specifically, while higher levels of baseline fibrinogen increased the odds of cognitive decline by 57% in females with AF (adjusted OR 1.57, 95% CI 1.36-1.81), we found no significant association among AF males (adjusted OR 1.13, 95% CI 0.99-1.30). This is potentially unsurprising, as dementia disproportionately affects females and studies have shown that females have faster rates of cognitive decline (147,148) and greater neuropathology (149–151) when compared to males. Although males are more likely to develop AF, AF females have a higher risk of thromboembolism and worse AF-related outcomes (24,152). Critically, sex-specific inflammatory differences have been reported as well where females had significantly higher levels of inflammatory markers, such as CRP and fibrinogen, compared to males (153,154). Contrary to the findings in the CHS cohort, sex was a significant effect modifier of the association between CRP and cognitive decline in the REGARDS cohort where sex-stratified results demonstrated the opposite effect. While AF males with elevated levels of CRP had higher odds of cognitive decline (adjusted OR 1.09, 95% CI 1.01-1.17), no association was found among females (adjusted OR 1.04, 95% CI 0.97-1.12). This is not consistent with previous studies reporting an association between CRP and cognitive decline only among females

(155,156); however, these analyses were not conducted in an AF population. As sex hormones have been previously shown to influence the concentration of plasma CRP and fibrinogen (157,158), biological sex-specific differences may underlie these effects. However, when excluding those with incident stroke in the REGARDS cohort, sex-specific associations were no longer significant. In post-hoc analyses in the REGARDS cohort, men were more likely to have incident stroke over follow-up (11.5% versus 9.7%, $p = 0.2$). This suggests that incident stroke may be responsible for the observed sex-specific effect rather than true biological differences. We also found that females in the CHS cohort were more likely to have incident stroke (22.0% versus 18.2%, $p = 0.007$). Although, when excluding those with incident stroke in sensitivity analyses, the sex-specific effects remained significant providing evidence for an effect of fibrinogen on cognitive decline in AF females in the absence of stroke. Sex-specific differences in performance on neurocognitive batteries may also account for the observed associations, as previous studies have highlighted that males tend to score higher on visuospatial tasks and females tend to have higher scores on tests of verbal memory (159–161). However, given evidence for widespread sex differences in both the concentrations and effects of multiple inflammatory markers on CVD risk (162,163), future work should continue to consider sex in the evaluation of inflammatory-mediated BBB dysfunction.

We also showed that in AF, the APOE Ɛ4 allele interacted with inflammatory markers to increase the odds of cognitive decline. Notably, however, the opposite was observed in the non-AF cohort, where the APOE Ɛ4 allele interacted with inflammatory markers, decreasing the risk of cognitive decline. As the APOE Ɛ4 allele is a major genetic risk factor for AD (164,165) and considering we controlled for important vascular risk factors and comorbidities, we suspect that the interactions observed in the non-AF cohort would demonstrate the modifying effects of APOE

genotype in pure AD pathology. Whereas in the AF cohort, those with the APOE Ɛ4 allele may have both vascular and AD pathologies contributing to cognitive decline, suggesting that inflammatory pathways may play a larger role in cognitive risk for those with vascular and mixed dementia. This is supported by findings reporting associations of inflammatory markers with VaD, but not AD or all-cause dementia (166,167). A previous study using data from the CHS showed that APOE Ɛ4 modulated baseline CRP and was notably associated with lower CRP levels (168). The APOE Ɛ4 allele has also been shown to interact with other cardiovascular diseases and substantially increase the risk of cognitive decline, compared to those without the allele (169). Further, studies have shown that the APOE Ɛ4 allele increases BBB permeability (170), impairs Aβ clearance from the brain (171), and may exacerbate neuroinflammatory responses (172,173). To our knowledge, previous studies have not investigated the modifying effect of APOE genotype on inflammatory-mediated cognitive decline with respect to AF status. However, prospective studies in the general population have found that higher levels of CRP were associated with an increased risk of cognitive decline and AD only among APOE Ɛ4 carriers (174,175) and particularly in the absence of cardiovascular disease (176). Although, this analysis did not exclude those with AF (176). While animal models have more clearly delineated the specific pathways by which APOE Ɛ4 increases dementia susceptibility, more work is needed to determine how this translates to the clinical setting, and whether this differs by dementia subtype and AF status. While there are some inconsistencies in the current literature, the modifying role of APOE Ɛ4 is clear and could help identify a high-risk group for cognitive decline.

In sensitivity analyses excluding those with incident stroke, CRP was the only marker that was no longer significantly associated with cognitive decline among those with AF. However, the association did remain significant among AF participants with the APOE Ɛ4 allele. Of the three

inflammatory markers, CRP displayed the weakest association with cognitive decline. Having said that, the non-significance of the association in a smaller stroke-free population is not surprising. The association between fibrinogen and cognitive decline among females was attenuated but remained significant. This was expected as, in addition to its role in the inflammatory response, fibrinogen is also a coagulation factor responsible for the maintenance of hemostasis and previous studies have reported positive associations of fibrinogen with stroke (177,178) and worse post-stroke outcomes (179). Based on our findings and previous longitudinal studies that have more consistently found significant associations of elevated IL-6 and fibrinogen with cognitive decline (154,180–182) and dementia (166,183), IL-6 and fibrinogen may be more sensitive markers of inflammatory-mediated BBB dysfunction. Future work should continue to carefully consider the mediating role of incident stroke, TIA, and SCI.

6.2 Sensitivity and Specificity of Peripheral Inflammatory Markers

In the CHS cohort we also investigated these associations in a non-AF population and found significant associations of inflammatory markers with cognitive decline, highlighting that inflammatory-mediated BBB dysfunction may not be unique to AF. Growing evidence supports inflammation and BBB dysfunction as a potentially important contributor to neurodegeneration and dementia, irrespective of AF status (9–11,51,102,106,112–114). In addition, these findings are consistent with previous prospective studies reporting that higher levels of these inflammatory markers were associated with an increased risk of dementia (120,166,167,184) and cognitive decline (154,180–182,185). However, this may also signify that these biomarkers do not accurately reflect BBB dysfunction and central inflammation. A major limitation of screening for peripherally circulating inflammatory markers, such as fibrinogen, CRP, and IL-6, is that these markers do not distinguish peripheral from CNS inflammation. Critically, a recent study showed that IL-6

signaling induces BBB dysfunction more strongly on the CNS side of the barrier than the vascular side, highlighting that central concentrations of these markers are more sensitive to BBB dysfunction (122). As CRP, IL-6, and fibrinogen are markers that play a wide range of functions in various inflammatory states (99,186), the observed associations may be due to residual confounding or confounding by uncontrolled factors, such as infection. This highlights the critical need to identify feasible biomarkers sensitive and specific to BBB dysfunction.

6.2.1 Potential novel marker of central inflammation and BBB dysfunction

Central inflammation leads to the activation of microglia which is pathogenically related to neurodegenerative processes (90). Activated microglia release cytokines and ROS, which can further contribute to BBB impairment and neurodegeneration (13). There is a critical need for new microglial-specific markers that can rapidly screen for central inflammation and BBB permeability to test this potential mechanistic pathway between AF and dementia. Increasing evidence shows that extracellular vesicles (EVs), released from microglia in response to inflammation, are potential brain inflammation-specific biomarkers as they can easily cross the BBB and be detected in the blood (187). Critically, EVs also contain cell-surface markers that are unique to their cell of origin, representing a potential blood-based marker of BBB permeability (187). Current analyses of BBB permeability rely on expensive imaging techniques and invasive lumbar punctures for the detection of biomarkers in the cerebrospinal fluid (187). However, the detection of these markers in blood-based EVs originating from activated microglia, would confirm their leakage into the CNS as a consequence of the loss of BBB integrity. A validated blood-based marker of BBB dysfunction would represent a significant breakthrough and a feasible way to further investigate inflammatory-mediated BBB dysfunction in large samples. This bioassay is currently under development and validation by our combined interdisciplinary research team.

6.3 Strengths

Our research was strengthened by the use of two large, well-characterized, population-based cohorts with median follow-up periods of approximately 9 years. The prospective design allowed us to investigate the temporal relationship between inflammation and cognition, providing evidence in favour of causality. As highlighted in *Chapter 2.4* and *Chapter 2.5*, there are multiple shared confounding factors and complex interrelationships between AF, inflammation, and cognitive impairment. In both cohorts, we adjusted for many of these potential confounders and investigated these associations in the absence of incident stroke. We were also able to investigate the modifying role of sex and APOE genotype. Future studies should continue to carefully control for confounding variables and distinguish them from intermediates (such as incident stroke, TIA, and SCI) and effect modifiers (such as sex and APOE genotype). Robust measures of these variables are critical to limit residual confounding. Furthermore, a mediation analysis is warranted to determine whether incident stroke, TIA, and SCI are intermediates on the causal pathway. As repeated measures of inflammatory markers were collected in the CHS cohort we were able to model exposure variables as time-varying in primary analyses, capturing fluctuating or chronically elevated levels of inflammatory makers over time. Longitudinal studies should aim to collect repeated measures of inflammatory markers to distinguish an acute-phase response, which has been shown to be initially neuroprotective (188), from a chronic response.

6.4 Limitations

There were also several limitations to our studies. First, subjects with missing cognitive scores may have been more likely to be lost to follow-up due to progressive cognitive decline, introducing selection bias and biasing our results towards the null. Second, in both AF populations, our analyses were underpowered. Third, the CHS study included both prevalent and incident AF cases,

reducing our ability to establish a temporal association and shortening the length of time between incident AF and dementia diagnosis. Fourth, our outcome measures were limited to all-cause dementia and global measures of cognitive function. Investigations of these associations by dementia subtype and domain-specific cognitive measures specific to the clinical presentation of dementia (i.e., executive function and memory) may provide greater insights. Particularly for the assessment of cognition, robust outcome ascertainment should be a focal point for future studies. As seen in the CHS study, specific strategies can be used to avoid loss-to-follow-up of dementia cases (for example, proxy interviews and retrieval of medical records). Fifth, incident stroke, TIA, and silent cerebral infarcts may be intermediates on the causal pathway accounting for the observed associations. However, in sensitivity analyses excluding those with incident stroke many associations remained significant. It is also important to note, that if the inflammatory markers tested in the present analyses change relative to either anticoagulation treatment initiation or cessation, there may be the potential for residual bias if treatment both confounds and mediates marker changes over time. Methods to address this residual bias using a g-estimation framework may be considered if sufficient data is available (189). Finally, as we have shown, these plasma-based inflammatory markers may not accurately reflect central inflammation and BBB dysfunction. We hypothesize that future studies using more sensitive markers of central inflammation and BBB dysfunction, as suggested above, will demonstrate inflammatory-mediated BBB dysfunction as an important contributing pathway linking AF to dementia.

6.5 Future Directions and Knowledge Translation Potential

As there is a limited amount of existing knowledge on the contributing pathways linking AF to dementia, many questions remain unanswered. Given the complex nature and long preclinical stage of dementia and the failure to find a disease-modifying drug to date (21), it is becoming

increasingly clear that an early tailored approach is required (21,55). Critically, survival with AF is increasing owing to improved therapies reducing the risk of stroke, mortality, and other AF-related outcomes (190,191). Evidence also suggests that those with the longest history of AF have the highest risk of dementia (61). With that said, AF patients may be highly amenable to an early targeted approach to prevent dementia (192). Our research provided an important first step to better understand the pathophysiological pathways linking AF to dementia and highlights important considerations for future research. This is a novel area of research with promising opportunities for future work. First, our findings provide some indication that inflammation occurs early in disease progression, increasing the promise that these markers could enable early detection and intervention of dementia, when disease modification is most likely (55). Future research is needed to determine the accuracy and predictive utility of these biomarkers in the AF population, as well as their optimal timing for measurement. Efforts should be made to derive clinical prediction models to estimate the utility of these markers (both peripheral and novel central biomarkers) for the prediction of dementia. Further, AUC-ROC analyses would demonstrate the discriminative performance of these markers for the diagnosis of dementia. Based on our findings, sex and APOE genotype should be integrated into these analyses. This research could greatly improve risk stratification and screening tools for dementia in AF patients (13). Further, whether these blood-based markers would be a feasible and minimally invasive alternative to current measures of BBB permeability remains unknown (55). Validation studies are required to compare these markers to current gold standard measures of BBB permeability. Second, while we primarily investigated the association of these markers with clinical presentations of dementia, it is becoming increasingly clear that subclinical changes are important markers for early detection and disease progression (55,193) and may be related to AF (84) and inflammation (168). However, more research is needed

to better understand the significance of preclinical pathology on neuroimaging as it relates to these markers and dementia risk in AF. Finally, in *Chapter 2.4* we summarized the possible contributing pathophysiological pathways linking AF to dementia. While we provided evidence for inflammatory-mediated BBB dysfunction, independent of risk factors shared by both AF and dementia, dementia is a multifactorial disease and it is likely that multiple etiologies are responsible (55). Inflammatory-mediated BBB dysfunction alone may not be sufficient to cause dementia. More work is needed to clarify the relative contribution of these pathways to dementia risk in AF.

6.6 Conclusion

A better understanding of the contributing pathophysiological pathways linking AF to dementia, independent of stroke, could enable better screening and risk stratification for improved clinical decision-making and highlight important therapeutic targets in the AF population. We aimed to examine inflammatory-mediated BBB dysfunction as a contributing pathway linking AF with cognitive decline and dementia. Our findings showed that elevated levels of inflammatory markers shown to influence BBB integrity were associated with cognitive decline but not dementia in two prospective AF populations. While these findings provide preliminary evidence for inflammatory-mediated cognitive decline in AF, future research using more sensitive markers of central infiltration are needed to determine the mediating role of BBB dysfunction in AF-related dementia risk. Further, as sex and APOE genotype were significant effect modifiers of the observed associations, these factors should be included in future research.

References

1. Wolf PA, Abbott RD, Kannel WB. Atrial Fibrillation as an Independent Risk Factor for Stroke: The Framingham Study. Stroke. 1991 Aug;22(8):983–8.

2. Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. Int J Stroke. 2020;1747493019897870–1747493019897870.

3. Canadian Institute for Health Information. Dementia in Canada: Summary [Internet]. [cited 2021 Jan 30]. Available from: https://www.cihi.ca/en/dementia-in-canada/dementia-in-canada-summary

4. World Health Organization. Dementia [Internet]. [cited 2021 Nov 17]. Available from: https://www.who.int/news-room/fact-sheets/detail/dementia

5. Kuźma E, Lourida I, Moore SF, Levine DA, Ukoumunne OC, Llewellyn DJ. Stroke and dementia risk: A systematic review and meta-analysis. Alzheimers Dement J Alzheimers Assoc. 2018 Nov;14(11):1416–26.

6. Kalantarian S, Stern TA, Mansour M, Ruskin JN. Cognitive Impairment Associated With Atrial Fibrillation. Ann Intern Med. 2013 Mar 5;158(5_Part_1):338–46.

7. Madhavan M, Graff-Radford J, Piccini JP, Gersh BJ. Cognitive dysfunction in atrial fibrillation. Nat Rev Cardiol. 2018 Dec;15(12):744–56.

8. Phillips KP. Role of Inflammation in Initiation and Perpetuation of Atrial Fibrillation: A Systematic Review of the Published Data. J Atr Fibrillation. 2013 Oct 31;6(3):935.

9. McGeer PL, Rogers J, McGeer EG. Inflammation, Antiinflammatory Agents, and Alzheimer's Disease: The Last 22 Years. J Alzheimers Dis JAD. 2016 Oct 4;54(3):853–7.

10. Holmes C. Review: systemic inflammation and Alzheimer's disease. Neuropathol Appl Neurobiol. 2013 Feb;39(1):51–68.

11. Koizumi K, Wang G, Park L. Endothelial Dysfunction and Amyloid-β-Induced Neurovascular Alterations. Cell Mol Neurobiol. 2016 Mar 1;36(2):155–65.

12. Hussain B, Fang C, Chang J. Blood–Brain Barrier Breakdown: An Emerging Biomarker of Cognitive Impairment in Normal Aging and Dementia. Front Neurosci. 2021;15:978.

13. Aryal, Ritambhara, Patabendige, Adjanie. Blood–brain barrier disruption in atrial fibrillation: a potential contributor to the increased risk of dementia and worsening of stroke outcomes? Open Biol. 11(4).

14. Ding WY, Gupta D, Lip GYH. Atrial fibrillation and the prothrombotic state: revisiting Virchow's triad in 2020. Heart. 2020 Oct 1;106(19):1463–8.

15. Crandall MA, Horne BD, Day JD, Anderson JL, Muhlestein JB, Crandall BG, et al. Atrial Fibrillation and CHADS2 Risk Factors are Associated with Highly Sensitive C-Reactive Protein Incrementally and Independently. Pacing Clin Electrophysiol. 2009;32(5):648–52.

16. Anderson JL, Allen Maycock CA, Lappé DL, Crandall BG, Horne BD, Bair TL, et al. Frequency of elevation of C-reactive protein in atrial fibrillation. Am J Cardiol. 2004 Nov 15;94(10):1255–9.

17. Guo Y, Lip GYH, Apostolakis S. Inflammation in Atrial Fibrillation. J Am Coll Cardiol. 2012 Dec 4;60(22):2263–70.

18. Mukamal KJ, Tolstrup JS, Friberg J, Grønbaek M, Jensen G. Fibrinogen and Albumin Levels and Risk of Atrial Fibrillation in Men and Women (the Copenhagen City Heart Study). Am J Cardiol. 2006 Jul 1;98(1):75–81.

19. Hsuchou H, Kastin AJ, Mishra PK, Pan W. C-reactive protein increases BBB permeability: implications for obesity and neuroinflammation. Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol. 2012;30(5):1109–19.

20. Rochfort KD, Collins LE, Murphy RP, Cummins PM. Downregulation of blood-brain barrier phenotype by proinflammatory cytokines involves NADPH oxidase-dependent ROS generation: consequences for interendothelial adherens and tight junctions. PloS One. 2014;9(7):e101815.

21. Yiannopoulou KG, Anastasiou AI, Zachariou V, Pelidou SH. Reasons for Failed Trials of Disease-Modifying Treatments for Alzheimer Disease and Their Contribution in Recent Research. Biomedicines. 2019 Dec 9;7(4):97.

22. Friberg L, Rosenqvist M. Less dementia with oral anticoagulation in atrial fibrillation. Eur Heart J. 2018 Feb 7;39(6):453–60.

23. John Hopkins. Atrial Fibrillation [Internet]. [cited 2021 Oct 23]. Available from: https://www.hopkinsmedicine.org/health/conditions-and-diseases/atrial-fibrillation

24. Kowey PR, Naccarelli GV. Atrial fibrillation. New York: Marcel Dekker; 2005. xvii+365. (Fundamental and clinical cardiology ; v. 49).

25. Benjamin EJ, Levy D, Vaziri SM, D'Agostino RB, Belanger AJ, Wolf PA. Independent risk factors for atrial fibrillation in a population-based cohort. The Framingham Heart Study. JAMA. 1994 Mar 16;271(11):840–4.

26. Psaty BM, Manolio TA, Kuller LH, Kronmal RA, Cushman M, Fried LP, et al. Incidence of and risk factors for atrial fibrillation in older adults. Circulation. 1997 Oct 7;96(7):2455–61.

27. Hindricks G, Potpara T, Dagres N, Arbelo E, Bax JJ, Blomström-Lundqvist C, et al. 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): The

Task Force for the diagnosis and management of atrial fibrillation of the European Society of Cardiology (ESC) Developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. Eur Heart J. 2021 Feb 1;42(5):373–498.

28. Heeringa J, van der Kuip DAM, Hofman A, Kors JA, van Herpen G, Stricker BHC, et al. Prevalence, incidence and lifetime risk of atrial fibrillation: the Rotterdam study. Eur Heart J. 2006 Apr;27(8):949–53.

29. Huxley RR, Lopez FL, Folsom AR, Agarwal SK, Loehr LR, Soliman EZ, et al. Absolute and attributable risks of atrial fibrillation in relation to optimal and borderline risk factors: the Atherosclerosis Risk in Communities (ARIC) study. Circulation. 2011 Apr 12;123(14):1501–8.

30. Schnabel RB, Yin X, Gona P, Larson MG, Beiser AS, McManus DD, et al. 50 year trends in atrial fibrillation prevalence, incidence, risk factors, and mortality in the Framingham Heart Study: a cohort study. The Lancet. 2015 Jul 11;386(9989):154–62.

31. Iwasaki Y ki, Nishida K, Kato T, Nattel S. Atrial Fibrillation Pathophysiology. Circulation. 2011 Nov 15;124(20):2264–74.

32. Wijesurendra RS, Casadei B. Mechanisms of atrial fibrillation. Heart. 2019 Dec 1;105(24):1860–7.

33. Yap YG. Essentials of atrial fibrillation. [Revised edition]. London: Springer Healthcare; 2014.

34. Crandall MA, Bradley DJ, Packer DL, Asirvatham SJ. Contemporary Management of Atrial Fibrillation: Update on Anticoagulation and Invasive Management Strategies. Mayo Clin Proc. 2009 Jul;84(7):643–62.

35. Lip GY, Hee FL. Paroxysmal atrial fibrillation. QJM Mon J Assoc Physicians. 2001 Dec;94(12):665–78.

36. Sposato LA, Cipriano LE, Saposnik G, Vargas ER, Riccio PM, Hachinski V. Diagnosis of atrial fibrillation after stroke and transient ischaemic attack: a systematic review and meta-analysis. Lancet Neurol. 2015 Apr;14(4):377–87.

37. Jaakkola J, Mustonen P, Kiviniemi T, Hartikainen JEK, Palomaki A, Hartikainen P, et al. Stroke as the First Manifestation of Atrial Fibrillation. PLoS ONE. 2016 Dec 9;11(12):e0168010–e0168010.

38. Wijffels MCEF, Kirchhof CJHJ, Dorland R, Allessie MA. Atrial Fibrillation Begets Atrial Fibrillation. Circulation. 1995 Oct 1;92(7):1954–68.

39. Kornej J, Börschel CS, Benjamin EJ, Schnabel RB. Epidemiology of Atrial Fibrillation in the 21st Century. Circ Res. 2020 Jun 19;127(1):4–20.

40. Sankaranarayanan R, Kirkwood G, Visweswariah R, Fox DJ. How does Chronic Atrial Fibrillation Influence Mortality in the Modern Treatment Era? Curr Cardiol Rev. 2015 Aug;11(3):190–8.

41. Odutayo A, Wong CX, Hsiao AJ, Hopewell S, Altman DG, Emdin CA. Atrial fibrillation and risks of cardiovascular disease, renal disease, and death: systematic review and meta-analysis. BMJ. 2016 Sep 6;354:i4482.

42. World Health Organization. The top 10 causes of death [Internet]. [cited 2021 Oct 25]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death

43. Jørgensen HS, Nakayama H, Reith J, Raaschou HO, Olsen TS. Acute stroke with atrial fibrillation. The Copenhagen Stroke Study. Stroke. 1996 Oct;27(10):1765–9.

44. Lin HJ, Wolf PA, Kelly-Hayes M, Beiser AS, Kase CS, Benjamin EJ, et al. Stroke severity in atrial fibrillation. The Framingham Study. Stroke. 1996 Oct;27(10):1760–4.

45. Penado S, Cano M, Acha O, Hernández JL, Riancho JA. Stroke severity in patients with atrial fibrillation. Am J Med. 2002 May 1;112(7):572–4.

46. Hayden DT, Hannon N, Callaly E, Ní Chróinín D, Horgan G, Kyne L, et al. Rates and Determinants of 5-Year Outcomes After Atrial Fibrillation–Related Stroke. Stroke. 2015 Dec 1;46(12):3488–93.

47. Saglietto A, Matta M, Gaita F, Jacobs V, Bunch TJ, Anselmino M. Stroke-independent contribution of atrial fibrillation to dementia: a meta-analysis. Open Heart. 2019;6(1):e000984.

48. Chambers LW, Bancej C, McDowell I. Prevalence and Monetary Costs of Dementia in Canada. Alzheimer Soc Can. 2016;70.

49. Joseph F. Quinn. Dementia. John Wiley & Sons, Ltd; 2014.

50. Wolters FJ, Ikram MA. Epidemiology of Vascular Dementia. Arterioscler Thromb Vasc Biol. 2019 Aug 1;39(8):1542–9.

51. Snyder HM, Corriveau RA, Craft S, Faber JE, Greenberg SM, Knopman D, et al. Vascular contributions to cognitive impairment and dementia including Alzheimer's disease. Alzheimers Dement. 2015 Jun 1;11(6):710–7.

52. Schneider, Julie A., Arvanitakis, Zoe, Bang, Woojeong, Bennett, David A. Mixed brain pathologies account for most dementia cases in community-dwelling older persons SYMBOL. Neurology. 69(24):2197–204.

53. James BD, Bennett DA, Boyle PA, Leurgans S, Schneider JA. Dementia From Alzheimer Disease and Mixed Pathologies in the Oldest Old. JAMA. 2012 May 2;307(17):1798–800.

54. Schneider JA, Arvanitakis Z, Leurgans SE, Bennett DA. The Neuropathology of Probable Alzheimer's Disease and Mild Cognitive Impairment. Ann Neurol. 2009 Aug;66(2):200–8.

55. Counts SE, Ikonomovic MD, Mercado N, Vega IE, Mufson EJ. Biomarkers for the Early Detection and Progression of Alzheimer's Disease. Neurotherapeutics. 2017 Jan 1;14(1):35–53.

56. Lang L, Clifford A, Wei L, Zhang D, Leung D, Augustine G, et al. Prevalence and determinants of undetected dementia in the community: a systematic literature review and a meta-analysis. BMJ Open. 2017 Feb 3;7(2):e011146.

57. Kametani F, Hasegawa M. Reconsideration of Amyloid Hypothesis and Tau Hypothesis in Alzheimer's Disease. Front Neurosci. 2018 Jan 30;12:25.

58. Raschetti R, Maggini M, Sorrentino GC, Martini N, Caffari B, Vanacore N. A cohort study of effectiveness of acetylcholinesterase inhibitors in Alzheimer's disease. Eur J Clin Pharmacol. 2005 Jul 1;61(5):361–8.

59. Marucci G, Buccioni M, Ben DD, Lambertucci C, Volpini R, Amenta F. Efficacy of acetylcholinesterase inhibitors in Alzheimer's disease. Neuropharmacology. 2021 Jun 1;190:108352.

60. Bunch TJ, Weiss JP, Crandall BG, May HT, Bair TL, Osborn JS, et al. Atrial fibrillation is independently associated with senile, vascular, and Alzheimer's dementia. Heart Rhythm. 2010 Apr 1;7(4):433–7.

61. de Bruijn RFAG, Heeringa J, Wolters FJ, Franco OH, Stricker BHC, Hofman A, et al. Association Between Atrial Fibrillation and Dementia in the General Population. JAMA Neurol. 2015;72(11):1–7.

62. Marzona I, O'Donnell M, Teo K, Gao P, Anderson C, Bosch J, et al. Increased risk of cognitive and functional decline in patients with atrial fibrillation: results of the ONTARGET and TRANSCEND studies. CMAJ Can Med Assoc J J Assoc Medicale Can. 2012 Apr 3;184(6):E329-336.

63. Thacker EL, McKnight B, Psaty BM, Longstreth WT, Sitlani CM, Dublin S, et al. Atrial fibrillation and cognitive decline: a longitudinal cohort study. Neurology. 2013 Jul 9;81(2):119–25.

64. Chen LY, Lopez FL, Gottesman RF, Huxley RR, Agarwal SK, Loehr L, et al. Atrial fibrillation and cognitive decline-the role of subclinical cerebral infarcts: the atherosclerosis risk in communities study. Stroke. 2014 Sep;45(9):2568–74.

65. Graves KG, May HT, Jacobs V, Bair TL, Stevens SM, Woller SC, et al. Atrial fibrillation incrementally increases dementia risk across all CHADS2 and CHA2DS2VASc strata in patients receiving long-term warfarin. Am Heart J. 2017 Jun 1;188:93–8.

66. Liao JN, Chao TF, Liu CJ, Wang KL, Chen SJ, Tuan TC, et al. Risk and prediction of dementia in patients with atrial fibrillation--a nationwide population-based cohort study. Int J Cardiol. 2015 Nov 15;199:25–30.

67. OTT A, BRETELER MMB, DE BRUYNE MC, VAN HARSKAMP F, GROBBEE DE, HOFMAN A. Atrial fibrillation and dementia in a population-based study: The Rotterdam study. Stroke 1970. 1997;28(2):316–21.

68. Peters R, Poulter R, Beckett N, Forette F, Fagard R, Potter J, et al. Cardiovascular and biochemical risk factors for incident dementia in the Hypertension in the Very Elderly Trial. J Hypertens. 2009 Oct;27(10):2055–62.

69. Rastas S, Verkkoniemi A, Polvikoski T, Juva K, Niinistö L, Mattila K, et al. Atrial Fibrillation, Stroke, and Cognition. Stroke. 2007 May 1;38(5):1454–60.

70. Marengoni A, Qiu C, Winblad B, Fratiglioni L. Atrial fibrillation, stroke and dementia in the very old: A population-based study. Neurobiol Aging. 2011 Jul 1;32(7):1336–7.

71. Mukamal KJ, Kuller LH, Fitzpatrick AL, Longstreth J WT, Mittleman MA, Siscovick DS. Prospective Study of Alcohol Consumption and Risk of Dementia in Older Adults. JAMA. 2003 Mar 19;289(11):1405–13.

72. Mukamal KJ, Tolstrup JS, Friberg J, Jensen G, Grønbæk M. Alcohol Consumption and Risk of Atrial Fibrillation in Men and Women. Circulation. 2005 Sep 20;112(12):1736–42.

73. Skoog I, Nilsson L, Persson G, Lernfelt B, Landahl S, Palmertz B, et al. 15-year longitudinal study of blood pressure and dementia. The Lancet. 1996 Apr 27;347(9009):1141–5.

74. Cheng G, Huang C, Deng H, Wang H. Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies. Intern Med J. 2012;42(5):484–91.

75. Qiu C, Winblad B, Marengoni A, Klarin I, Fastbom J, Fratiglioni L. Heart Failure and Risk of Dementia and Alzheimer Disease: A Population-Based Cohort Study. Arch Intern Med. 2006 May 8;166(9):1003–8.

76. Alonso A, Lopez FL, Matsushita K, Loehr LR, Agarwal SK, Chen LY, et al. Chronic Kidney Disease Is Associated With the Incidence of Atrial Fibrillation. Circulation. 2011 Jun 28;123(25):2946–53.

77. Xu H, Garcia-Ptacek S, Trevisan M, Evans M, Lindholm B, Eriksdotter M, et al. Kidney Function, Kidney Function Decline, and the Risk of Dementia in Older Adults: A Registry-Based Study. Neurology. 2021 Jun 15;96(24):e2956–65.

78. de Bruijn RFAG, Heeringa J, Wolters FJ, Franco OH, Stricker BHC, Hofman A, et al. Association Between Atrial Fibrillation and Dementia in the General Population. JAMA Neurol. 2015 Nov;72(11):1288–94.

79. Kaski JC, Arrebola-Moreno AL. Inflammation and Thrombosis in Atrial Fibrillation. Rev Esp Cardiol Engl Ed. 2011 Jul 1;64(7):551–3.

80. Goldberg I, Auriel E, Russell D, Korczyn AD. Microembolism, silent brain infarcts and dementia. J Neurol Sci. 2012 Nov 15;322(1–2):250–3.

81. Vermeer SE, Prins ND, den Heijer T, Hofman A, Koudstaal PJ, Breteler MMB. Silent brain infarcts and the risk of dementia and cognitive decline. N Engl J Med. 2003 Mar 27;348(13):1215–22.

82. Rydén L, Sacuiu S, Wetterberg H, Najar J, Guo X, Kern S, et al. Atrial Fibrillation, Stroke, and Silent Cerebrovascular Disease: A Population-based MRI Study. Neurology. 2021 Oct 19;97(16):e1608–19.

83. Graff-Radford J, Madhavan M, Vemuri P, Rabinstein AA, Cha RH, Mielke MM, et al. Atrial fibrillation, cognitive impairment, and neuroimaging. Alzheimers Dement. 2016 Apr 1;12(4):391–8.

84. Stefansdottir H, Arnar DO, Aspelund T, Sigurdsson S, Jonsdottir MK, Hjaltason H, et al. Atrial fibrillation is associated with reduced brain volume and cognitive function independent of cerebral infarcts. Stroke. 2013 Apr;44(4):1020–5.

85. Farina E, Magni E, Ambrosini F, Manfredini R, Binda A, Sina C, et al. Neuropsychological deficits in asymptomatic atrial fibrillation. Acta Neurol Scand. 1997 Nov;96(5):310–6.

86. Gutierrez C, Blanchard DG. Diagnosis and Treatment of Atrial Fibrillation. Am Fam Physician. 2016 Sep 15;94(6):442–52.

87. Akoudad S, Wolters FJ, Viswanathan A, de Bruijn RF, van der Lugt A, Hofman A, et al. Association of Cerebral Microbleeds With Cognitive Decline and Dementia. JAMA Neurol. 2016 Aug 1;73(8):934–43.

88. Zlokovic BV, Gottesman RF, Bernstein KE, Seshadri S, McKee A, Snyder H, et al. Vascular contributions to cognitive impairment and dementia (VCID): A report from the 2018 National Heart, Lung, and Blood Institute and National Institute of Neurological Disorders and Stroke Workshop. Alzheimers Dement. 2020 Dec 1;16(12):1714–33.

89. Aldrugh S, Sardana M, Henninger N, Saczynski JS, McManus DD. Atrial fibrillation, cognition and dementia: A review. J Cardiovasc Electrophysiol. 2017;28(8):958–65.

90. Martins GL, Duarte RCF, Mukhamedyarov MA, Palotás A, Ferreira CN, Reis HJ. Inflammatory and Infectious Processes Serve as Links between Atrial Fibrillation and Alzheimer's Disease. Int J Mol Sci. 2020 Jan;21(9):3226.

91. Gardarsdottir M, Sigurdsson S, Aspelund T, Rokita H, Launer LJ, Gudnason V, et al. Atrial fibrillation is associated with decreased total cerebral blood flow and brain perfusion. Europace. 2018 Aug;20(8):1252–8.

92. Manolio TA, Kronmal RA, Burke GL, Poirier V, O'Leary DH, Gardin JM, et al. Magnetic resonance abnormalities and cardiovascular disease in older adults. The Cardiovascular Health Study. Stroke. 1994 Feb 1;25(2):318–27.

93. Seshadri S, Wolf PA, Beiser A, Elias MF, Au R, Kase CS, et al. Stroke risk profile, brain volume, and cognitive function: The Framingham Offspring Study. Neurology. 2004 Nov 9;63(9):1591–9.

94. Shi Y, Thrippleton MJ, Makin SD, Marshall I, Geerlings MI, de Craen AJ, et al. Cerebral blood flow in small vessel disease: A systematic review and meta-analysis. J Cereb Blood Flow Metab. 2016 Oct 1;36(10):1653–67.

95. Jefferson AL, Himali JJ, Au R, Seshadri S, Decarli C, O'Donnell CJ, et al. Relation of left ventricular ejection fraction to cognitive aging (from the Framingham Heart Study). Am J Cardiol. 2011 Nov 1;108(9):1346–51.

96. Bunch TJ, Crandall BG, Weiss JP, May HT, Bair TL, Osborn JS, et al. Patients Treated with Catheter Ablation for Atrial Fibrillation Have Long-Term Rates of Death, Stroke, and Dementia Similar to Patients Without Atrial Fibrillation. J Cardiovasc Electrophysiol. 2011;22(8):839–45.

97. Kirchhof P, Camm AJ, Goette A, Brandes A, Eckardt L, Elvan A, et al. Early Rhythm-Control Therapy in Patients with Atrial Fibrillation. N Engl J Med. 2020 Oct 1;383(14):1305–16.

98. Chung MK, Shemanski L, Sherman DG, Greene HL, Hogan DB, Kellen JC, et al. Functional Status in Rate- Versus Rhythm-Control Strategies for Atrial Fibrillation. J Am Coll Cardiol. 2005 Nov 15;46(10):1891–9.

99. Castell JV, Gómez-Lechón MJ, David M, Andus T, Geiger T, Trullenque R, et al. Interleukin-6 is the major regulator of acute phase protein synthesis in adult human hepatocytes. FEBS Lett. 1989 Jan 2;242(2):237–9.

100. Harada M, Wagoner DRV, Nattel S. Role of Inflammation in Atrial Fibrillation Pathophysiology and Management. Circ J. 2015;79(3):495–502.

101. Elwood E, Lim Z, Naveed H, Galea I. The effect of systemic inflammation on human brain barrier function. Brain Behav Immun. 2017 May;62:35–40.

102. Lue LF, Brachova L, Civin WH, Rogers J. Inflammation, A beta deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration. J Neuropathol Exp Neurol. 1996 Oct;55(10):1083–8.

103. The Blood–Brain Barrier [Internet]. [cited 2021 Nov 5]. Available from: https://www-ncbi-nlm-nih-gov.proxy.bib.uottawa.ca/pmc/articles/PMC4292164/

104. Daneman R. The blood–brain barrier in health and disease. Ann Neurol. 2012;72(5):648–72.

105. Ouellette J, Lacoste B. From Neurodevelopmental to Neurodegenerative Disorders: The Vascular Continuum. Front Aging Neurosci [Internet]. 2021 [cited 2022 Jul 23];13. Available from: https://www.frontiersin.org/articles/10.3389/fnagi.2021.749026

106. Sweeney MD, Sagare AP, Zlokovic BV. Blood–brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol. 2018 Mar;14(3):133–50.

107. Daneman R, Prat A. The Blood–Brain Barrier. Cold Spring Harb Perspect Biol. 2015 Jan;7(1):a020412.

108. Van Dyken P, Lacoste B. Impact of Metabolic Syndrome on Neuroinflammation and the Blood–Brain Barrier. Front Neurosci [Internet]. 2018 [cited 2022 Jul 23];12. Available from: https://www.frontiersin.org/articles/10.3389/fnins.2018.00930

109. Freitas-Andrade M, Raman-Nair J, Lacoste B. Structural and Functional Remodeling of the Brain Vasculature Following Stroke. Front Physiol [Internet]. 2020 [cited 2022 Jul 23];11. Available from: https://www.frontiersin.org/articles/10.3389/fphys.2020.00948

110. Bachiller S, Jiménez-Ferrer I, Paulus A, Yang Y, Swanberg M, Deierborg T, et al. Microglia in Neurological Diseases: A Road Map to Brain-Disease Dependent-Inflammatory Response. Front Cell Neurosci [Internet]. 2018 [cited 2022 Jul 23];12. Available from: https://www.frontiersin.org/articles/10.3389/fncel.2018.00488

111. Barisano G, Montagne A, Kisler K, Schneider JA, Wardlaw JM, Zlokovic BV. Blood–brain barrier link to human cognitive impairment and Alzheimer's disease. Nat Cardiovasc Res. 2022 Feb;1(2):108–15.

112. Cai Z, Qiao PF, Wan CQ, Cai M, Zhou NK, Li Q. Role of Blood-Brain Barrier in Alzheimer's Disease. J Alzheimers Dis. 2018 Jan 1;63(4):1223–34.

113. Zenaro E, Piacentino G, Constantin G. The blood-brain barrier in Alzheimer's disease. Neurobiol Dis. 2017 Nov;107:41–56.

114. Nation DA, Sweeney MD, Montagne A, Sagare AP, D'Orazio LM, Pachicano M, et al. Blood–brain barrier breakdown is an early biomarker of human cognitive dysfunction. Nat Med. 2019 Feb;25(2):270–6.

115. Liu C, Shi F, Chen Z, Yan S, Ding X, Lou M. Severe Blood-Brain Barrier Disruption in Cardioembolic Stroke. Front Neurol. 2018;9:55.

116. Galenko O, Jacobs V, Knight S, Bride D, Cutler MJ, Muhlestein JB, et al. Circulating Levels of Biomarkers of Cerebral Injury in Patients with Atrial Fibrillation. Am J Cardiol. 2019 Dec 1;124(11):1697–700.

117. Pun PBL, Lu J, Moochhala S. Involvement of ROS in BBB dysfunction. Free Radic Res. 2009 Jan 1;43(4):348–64.

118. Ryu JK, McLarnon JG. A leaky blood–brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. J Cell Mol Med. 2009;13(9a):2911–25.

119. Cortes-Canteli M, Paul J, Norris EH, Bronstein R, Ahn HJ, Zamolodchikov D, et al. Fibrinogen and β-amyloid association alters thrombosis and fibrinolysis: a possible contributing factor to Alzheimer's disease. Neuron. 2010 Jun 10;66(5):695–709.

120. van Oijen Marieke, Witteman Jacqueline C., Hofman Albert, Koudstaal Peter J., Breteler Monique M.B. Fibrinogen Is Associated With an Increased Risk of Alzheimer Disease and Vascular Dementia. Stroke. 2005 Dec 1;36(12):2637–41.

121. Xu G, Zhang H, Zhang S, Fan X, Liu X. Plasma fibrinogen is associated with cognitive decline and risk for dementia in patients with mild cognitive impairment. Int J Clin Pract. 2008 Jul;62(7):1070–5.

122. Takeshita Y, Fujikawa S, Serizawa K, Fujisawa M, Matsuo K, Nemoto J, et al. New BBB Model Reveals That IL-6 Blockade Suppressed the BBB Disorder, Preventing Onset of NMOSD. Neurol - Neuroimmunol Neuroinflammation [Internet]. 2021 Nov 1 [cited 2022 Mar 26];8(6). Available from: https://nn.neurology.org/content/8/6/e1076

123. PATIBANDLA PK, TYAGI N, DEAN WL, TYAGI SC, ROBERTS AM, LOMINADZE D. Fibrinogen Induces Alterations of Endothelial Cell Tight Junction Proteins. J Cell Physiol. 2009 Oct;221(1):195–203.

124. Paul J, Strickland S, Melchor JP. Fibrin deposition accelerates neurovascular damage and neuroinflammation in mouse models of Alzheimer's disease. J Exp Med [Internet]. 2007 [cited 2022 Mar 26];204(8). Available from: https://www.readcube.com/articles/10.1084%2Fjem.20070304

125. Howard VJ, Cushman M, Pulley L, Gomez CR, Go RC, Prineas RJ, et al. The reasons for geographic and racial differences in stroke study: objectives and design. Neuroepidemiology. 2005;25(3):135–43.

126. Howard VJ, Kleindorfer DO, Judd SE, McClure LA, Safford MM, Rhodes JD, et al. Disparities in stroke incidence contributing to disparities in stroke mortality. Ann Neurol. 2011;69(4):619–27.

127. Callahan CM, Unverzagt FW, Hui SL, Perkins AJ, Hendrie HC. Six-Item Screener to Identify Cognitive Impairment among Potential Subjects for Clinical Research. Med Care. 2002;40(9):771–81.

128. Fried LP, Borhani NO, Enright P, Furberg CD, Gardin JM, Kronmal RA, et al. The cardiovascular health study: Design and rationale. Ann Epidemiol. 1991 Feb 1;1(3):263–76.

129. Fitzpatrick AL, Kuller LH, Ives DG, Lopez OL, Jagust W, Breitner JCS, et al. Incidence and Prevalence of Dementia in the Cardiovascular Health Study. J Am Geriatr Soc. 2004;52(2):195–204.

130. Lopez OL, Kuller LH, Fitzpatrick A, Ives D, Becker JT, Beauchamp N. Evaluation of dementia in the cardiovascular health cognition study. Neuroepidemiology. 2003 Feb;22(1):1–12.

131. Lau B, Cole SR, Gange SJ. Competing risk regression models for epidemiologic data. Am J Epidemiol. 2009 Jul 15;170(2):244–56.

132. Austin PC, Lee DS, Fine JP. Introduction to the Analysis of Survival Data in the Presence of Competing Risks. Circulation. 2016 Feb 9;133(6):601–9.

133. Wardenaar K. Latent Class Growth Analysis and Growth Mixture Modeling using R: A tutorial for two R-packages and a comparison with Mplus. [Internet]. PsyArXiv; 2020 [cited 2022 Jan 16]. Available from: https://psyarxiv.com/m58wx/

134. Therneau TM. Modeling survival data: extending the Cox model. New York: Springer; 2000. (Statistics for biology and health).

135. Lee, Katherine J, Tilling, Kate M, Cornish, Rosie P, Little, Roderick J A, Bell, Melanie L, Goetghebeur, Els, et al. Framework for the treatment and reporting of missing data in observational studies: The Treatment And Reporting of Missing data in Observational Studies framework. J Clin Epidemiol. 2021 Jun 1;134:79–88.

136. Bryan RN, Manolio TA, Schertz LD, Jungreis C, Poirier VC, Elster AD, et al. A method for using MR to evaluate the effects of cardiovascular disease on the brain: the cardiovascular health study. Am J Neuroradiol. 1994 Oct 1;15(9):1625–33.

137. Jack CR, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner MW, et al. Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. Lancet Neurol. 2010 Jan;9(1):119.

138. Buchhave P, Minthon L, Zetterberg H, Wallin ÅK, Blennow K, Hansson O. Cerebrospinal Fluid Levels ofβ-Amyloid 1-42, but Not of Tau, Are Fully Changed Already 5 to 10 Years Before the Onset of Alzheimer Dementia. Arch Gen Psychiatry. 2012 Jan 1;69(1):98–106.

139. Engler H, Forsberg A, Almkvist O, Blomquist G, Larsson E, Savitcheva I, et al. Two-year follow-up of amyloid deposition in patients with Alzheimer's disease. Brain. 2006 Nov 1;129(11):2856–66.

140. Landau SM, Mintun MA, Joshi AD, Koeppe RA, Petersen RC, Aisen PS, et al. Amyloid Deposition, Hypometabolism, and Longitudinal Cognitive Decline. Ann Neurol. 2012 Oct;72(4):578–86.

141. Ingelsson M, Fukumoto H, Newell KL, Growdon JH, Hedley-Whyte ET, Frosch MP, et al. Early Abeta accumulation and progressive synaptic loss, gliosis, and tangle formation in AD brain. Neurology. 2004 Mar 23;62(6):925–31.

142. Monson NL, Ireland SJ, Ligocki AJ, Chen D, Rounds WH, Li M, et al. Elevated CNS inflammation in patients with preclinical Alzheimer's disease. J Cereb Blood Flow Metab. 2014 Jan;34(1):30–3.

143. Parbo P, Ismail R, Hansen KV, Amidi A, Mårup FH, Gottrup H, et al. Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. Brain J Neurol. 2017 Jul 1;140(7):2002–11.

144. Ismail R, Parbo P, Madsen LS, Hansen AK, Hansen KV, Schaldemose JL, et al. The relationships between neuroinflammation, beta-amyloid and tau deposition in Alzheimer's disease: a longitudinal PET study. J Neuroinflammation. 2020 May 6;17(1):151.

145. De Roeck EE, De Deyn PP, Dierckx E, Engelborghs S. Brief cognitive screening instruments for early detection of Alzheimer's disease: a systematic review. Alzheimers Res Ther. 2019 Feb 28;11(1):21.

146. Kim D, Yang PS, Joung B. Prevention of Dementia in Patients with Atrial Fibrillation. Korean Circ J. 2021 Mar 11;51(4):308–19.

147. Levine DA, Gross AL, Briceño EM, Tilton N, Giordani BJ, Sussman JB, et al. Sex Differences in Cognitive Decline Among US Adults. JAMA Netw Open. 2021 Feb 25;4(2):e210169.

148. Irvine K, Laws KR, Gale TM, Kondel TK. Greater cognitive deterioration in women than men with Alzheimer's disease: a meta analysis. J Clin Exp Neuropsychol. 2012;34(9):989–98.

149. Ardekani BA, Convit A, Bachman AH. Analysis of the MIRIAD Data Shows Sex Differences in Hippocampal Atrophy Progression. J Alzheimers Dis JAD. 2016;50(3):847–57.

150. Barnes LL, Wilson RS, Bienias JL, Schneider JA, Evans DA, Bennett DA. Sex differences in the clinical manifestations of Alzheimer disease pathology. Arch Gen Psychiatry. 2005 Jun;62(6):685–91.

151. Hua X, Hibar DP, Lee S, Toga AW, Jack CR, Weiner MW, et al. Sex and age differences in atrophic rates: an ADNI study with N=1368 MRI scans. Neurobiol Aging. 2010 Aug;31(8):1463–80.

152. Lip GYH, Laroche C, Boriani G, Cimaglia P, Dan GA, Santini M, et al. Sex-related differences in presentation, treatment, and outcome of patients with atrial fibrillation in Europe: a report from the Euro Observational Research Programme Pilot survey on Atrial Fibrillation. EP Eur. 2015 Jan 1;17(1):24–31.

153. Duarte-Guterman P, Albert AY, Inkster AM, Barha CK, Galea LAM, Alzheimer's Disease Neuroimaging Initiative. Inflammation in Alzheimer's Disease: Do Sex and APOE Matter? J Alzheimers Dis JAD. 2020;78(2):627–41.

154. Pyun JM, Ryoo N, Park YH, Kim S. Fibrinogen Levels and Cognitive Profile Differences in Patients with Mild Cognitive Impairment. Dement Geriatr Cogn Disord. 2021 Feb;49(5):489–96.

155. Watanabe Y, Kitamura K, Nakamura K, Sanpei K, Wakasugi M, Yokoseki A, et al. Elevated C-Reactive Protein Is Associated with Cognitive Decline in Outpatients of a General Hospital: The Project in Sado for Total Health (PROST). Dement Geriatr Cogn Disord EXTRA. 2016 Jan 19;6(1):10–9.

156. Kravitz BA, Corrada MM, Kawas CH. Elevated C-reactive protein levels are associated with prevalent dementia in the oldest-old. Alzheimers Dement J Alzheimers Assoc. 2009 Jul;5(4):318–23.

157. Cushman M, Legault C, Barrett-Connor E, Stefanick ML, Kessler C, Judd HL, et al. Effect of postmenopausal hormones on inflammation-sensitive proteins: the Postmenopausal Estrogen/Progestin Interventions (PEPI) Study. Circulation. 1999 Aug 17;100(7):717–22.

158. Folsom AR, Golden SH, Boland LL, Szklo M. Association of Endogenous Hormones with C-Reactive Protein, Fibrinogen, and White Blood Count in Post-Menopausal Women. Eur J Epidemiol. 2005;20(12):1015–22.

159. Andrew MK, Tierney MC. The puzzle of sex, gender and Alzheimer's disease: Why are women more often affected than men? Womens Health. 2018 Jan 1;14:1745506518817995.

160. Gerstorf D, Herlitz A, Smith J. Stability of Sex Differences in Cognition in Advanced Old Age: The Role of Education and Attrition. J Gerontol Ser B. 2006 Jul 1;61(4):P245–9.

161. van Hooren SAH, Valentijn AM, Bosma H, Ponds RWHM, van Boxtel MPJ, Jolles J. Cognitive Functioning in Healthy Older Adults Aged 64–81: A Cohort Study into the Effects of Age, Sex, and Education. Aging Neuropsychol Cogn. 2007 Jan 10;14(1):40–54.

162. Lau ES, Paniagua SM, Guseh JS, Bhambhani V, Zanni MV, Courchesne P, et al. Sex Differences in Circulating Biomarkers of Cardiovascular Disease. J Am Coll Cardiol. 2019 Sep 24;74(12):1543–53.

163. Li T, Wang F, Peng R, Pei S, Hou Z, Lu B, et al. Sex-related differences in the association between plasma fibrinogen and non-calcified or mixed coronary atherosclerotic plaques. Biol Sex Differ. 2018 Dec 5;9(1):51.

164. Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms, and therapy. Nat Rev Neurol. 2013 Feb;9(2):106–18.

165. Strittmatter WJ, Roses AD. Apolipoprotein E and Alzheimer disease. Proc Natl Acad Sci U S A. 1995 May 23;92(11):4725–7.

166. Engelhart MJ, Geerlings MI, Meijer J, Kiliaan A, Ruitenberg A, van Swieten JC, et al. Inflammatory Proteins in Plasma and the Risk of Dementia: The Rotterdam Study. Arch Neurol. 2004 May 1;61(5):668–72.

167. Hsu PF, Pan WH, Yip BS, Chen RCY, Cheng HM, Chuang SY. C-Reactive Protein Predicts Incidence of Dementia in an Elderly Asian Community Cohort. J Am Med Dir Assoc. 2017 Mar 1;18(3):277.e7-277.e11.

168. Corlier F, Hafzalla G, Faskowitz J, Kuller LH, Becker JT, Lopez OL, et al. Systemic inflammation as a predictor of brain aging: Contributions of physical activity, metabolic risk, and genetic risk. NeuroImage. 2018 May 15;172:118–29.

169. Haan MN, Shemanski L, Jagust WJ, Manolio TA, Kuller L. The role of APOE epsilon4 in modulating effects of other risk factors for cognitive decline in elderly persons. JAMA. 1999 Jul 7;282(1):40–6.

170. Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hulette CM, et al. Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. Neurobiol Aging. 2007 Jul;28(7):977–86.

171. Zlokovic BV. Cerebrovascular Effects of Apolipoprotein E: Implications for Alzheimer Disease. JAMA Neurol. 2013 Apr 1;70(4):440–4.

172. Kloske CM, Wilcock DM. The Important Interface Between Apolipoprotein E and Neuroinflammation in Alzheimer's Disease. Front Immunol [Internet]. 2020 [cited 2022 Apr 10];11. Available from: https://www.frontiersin.org/article/10.3389/fimmu.2020.00754

173. Guo L, LaDu MJ, Eldik LJ. A dual role for apolipoprotein E in neuroinflammation : Anti- and pro-inflammatory activity. J Mol Neurosci. 2004;23(3):205–12.

174. Noble JM, Manly JJ, Schupf N, Tang MX, Mayeux R, Luchsinger JA. Association of C-Reactive Protein With Cognitive Impairment. Arch Neurol. 2010 Jan 1;67(1):87–92.

175. Lima TAS, Adler AL, Minett T, Matthews FE, Brayne C, Marioni RE, et al. C-reactive protein, APOE genotype and longitudinal cognitive change in an older population. Age Ageing. 2014 Mar 1;43(2):289–92.

176. Tao Q, Ang TFA, DeCarli C, Auerbach SH, Devine S, Stein TD, et al. Association of Chronic Low-grade Inflammation With Risk of Alzheimer Disease in ApoE4 Carriers. JAMA Netw Open. 2018 Oct 19;1(6):e183597.

177. Peycheva M, Deneva T, Zahariev Z. The role of fibrinogen in acute ischaemic stroke. Neurol Neurochir Pol. 2021;55(1):74–80.

178. Samir GM, Khalil OA, Fawzy MS, Sadek AMEM. Study of Fibrinogen Level in Acute Ischemic Stroke Patients in Medical Intensive Care Unit. Egypt J Crit Care Med. 2020 Dec;7(2 and 3):51–6.

179. Lee SJ, Hong JM, Lee SE, Kang DR, Ovbiagele B, Demchuk AM, et al. Association of fibrinogen level with early neurological deterioration among acute ischemic stroke patients with diabetes. BMC Neurol. 2017 May 19;17(1):101.

180. Singh-Manoux A, Dugravot A, Brunner E, Kumari M, Shipley M, Elbaz A, et al. Interleukin-6 and C-reactive protein as predictors of cognitive decline in late midlife. Neurology. 2014 Aug 5;83(6):486–93.

181. Rafnsson SB, Deary IJ, Smith FB, Whiteman MC, Rumley A, Lowe GDO, et al. Cognitive Decline and Markers of Inflammation and Hemostasis: The Edinburgh Artery Study. J Am Geriatr Soc. 2007;55(5):700–7.

182. Economos A, Wright CB, Moon YP, Rundek T, Rabbani L, Paik MC, et al. Interleukin 6 Plasma Concentration Associates with Cognitive Decline: The Northern Manhattan Study. Neuroepidemiology. 2013;40(4):253–9.

183. van Oijen M, Witteman JC, Hofman A, Koudstaal PJ, Breteler MMB. Fibrinogen Is Associated With an Increased Risk of Alzheimer Disease and Vascular Dementia. Stroke. 2005 Dec;36(12):2637–41.

184. Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ. Early inflammation and dementia: A 25-year follow-up of the Honolulu-Asia aging study. Ann Neurol. 2002;52(2):168–74.

185. Yaffe K, Lindquist K, Penninx BW, Simonsick EM, Pahor M, Kritchevsky S, et al. Inflammatory markers and cognition in well-functioning African-American and white elders. Neurology. 2003 Jul 8;61(1):76–80.

186. Ansar W, Ghosh S. Clinical significance of C-reactive protein. Singapore: Springer; 2020.

187. Hornung S, Dutta S, Bitan G. CNS-Derived Blood Exosomes as a Promising Source of Biomarkers: Opportunities and Challenges. Front Mol Neurosci [Internet]. 2020 [cited 2021 Mar 21];13. Available from: https://www.frontiersin.org/articles/10.3389/fnmol.2020.00038/full

188. Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. Inflammation as a central mechanism in Alzheimer's disease. Alzheimers Dement Transl Res Clin Interv. 2018 Sep 6;4:575–90.

189. Daniel R m., Cousens S n., De Stavola B l., Kenward MG, Sterne J a. C. Methods for dealing with time-dependent confounding. Stat Med. 2013;32(9):1584–618.

190. Schnabel RB, Yin X, PhilimonGona, Larson MG, Beiser AS, McManus DD, et al. Fifty-Year Trends in Atrial Fibrillation Prevalence, Incidence, Risk Factors, and Mortality in the Community. Lancet Lond Engl. 2015 Jul 11;386(9989):154–62.

191. Asbach S, Olschewski M, Faber TS, Zehender M, Bode C, Brunner M. Mortality in patients with atrial fibrillation has significantly decreased during the last three decades: 35 years of follow-up in 1627 pacemaker patients. EP Eur. 2008 Apr 1;10(4):391–4.

192. Rivard L, Friberg L, Conen D, Healey JS, Berge T, Boriani G, et al. Atrial Fibrillation and Dementia: A Report From the AF-SCREEN International Collaboration. Circulation. 2022 Feb;145(5):392–409.

193. Staffaroni AM, Elahi FM, McDermott D, Marton K, Karageorgiou E, Sacco S, et al. Neuroimaging in Dementia. Semin Neurol. 2017 Oct;37(5):510–37.

194. Test Time-To-Event Data Cox PH, Equivalence | Power and Sample Size Calculators | HyLown [Internet]. [cited 2022 Feb 20]. Available from: http://powerandsamplesize.com/Calculators/Test-Time-To-Event-Data/Cox-PH-Equivalence

Appendix A. Study Designs

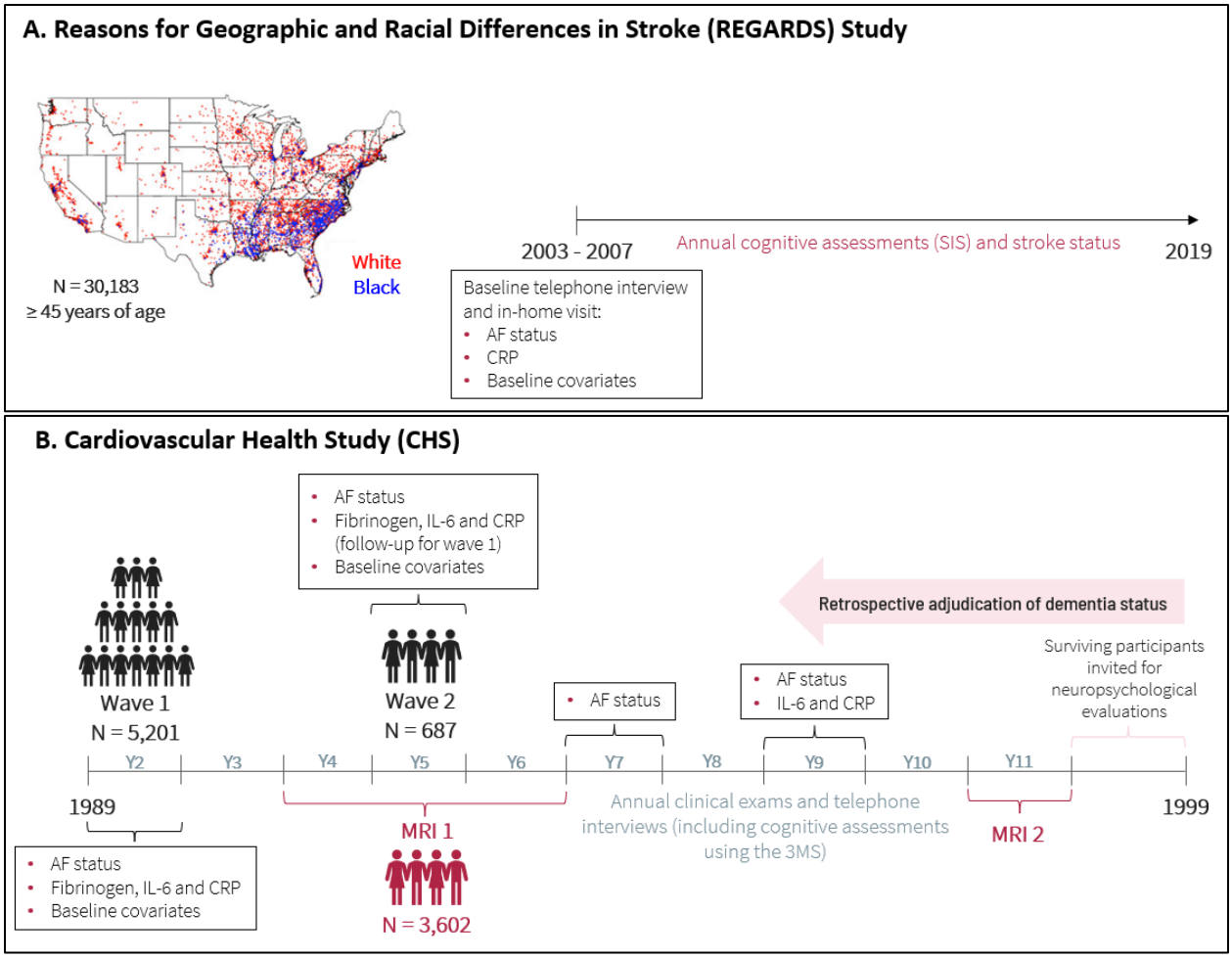


Figure A-1. Study Designs. A. REGARDS enrolled 30,183 Black and White adults aged ≥ 45 years in the U.S. Participants underwent a baseline telephone interview and in-home visit. Cognitive assessments were administered annually and stroke status was adjudicated at 6-month intervals. **B.** CHS enrolled 5,888 adults ≥ 65 years (5,201 in 1989-90 and 687 predominantly Black participants in 1992-93). Dementia status and onset was retrospectively adjudicated using available data among the subset who received an MRI (annual 3MS scores, MRI, medical record retrieval, physician or proxy interviews, and neuropsychological evaluations among surviving participants). SIS: six-item screener; AF: atrial fibrillation; CRP: C-reactive protein; IL-6: interleukin-6; 3MS: Modified mini-mental state examination.

Appendix B. Sample Size Calculations

To assess whether the available data are powered to support the proposed study, we used the equation outlined below (194).

$$n = \frac{1}{pA\ pB\ pE} \frac{z_{1-\alpha} + z_{1-\beta/2}}{\delta - |\ln(\theta)|}$$

where:

n is sample size

pE is the overall probability of the event occurring within the study period

pA and pB are the proportions of the sample size allotted to the two groups

α is Type I error

β is Type II error, meaning 1-β is power

δ is the testing margin

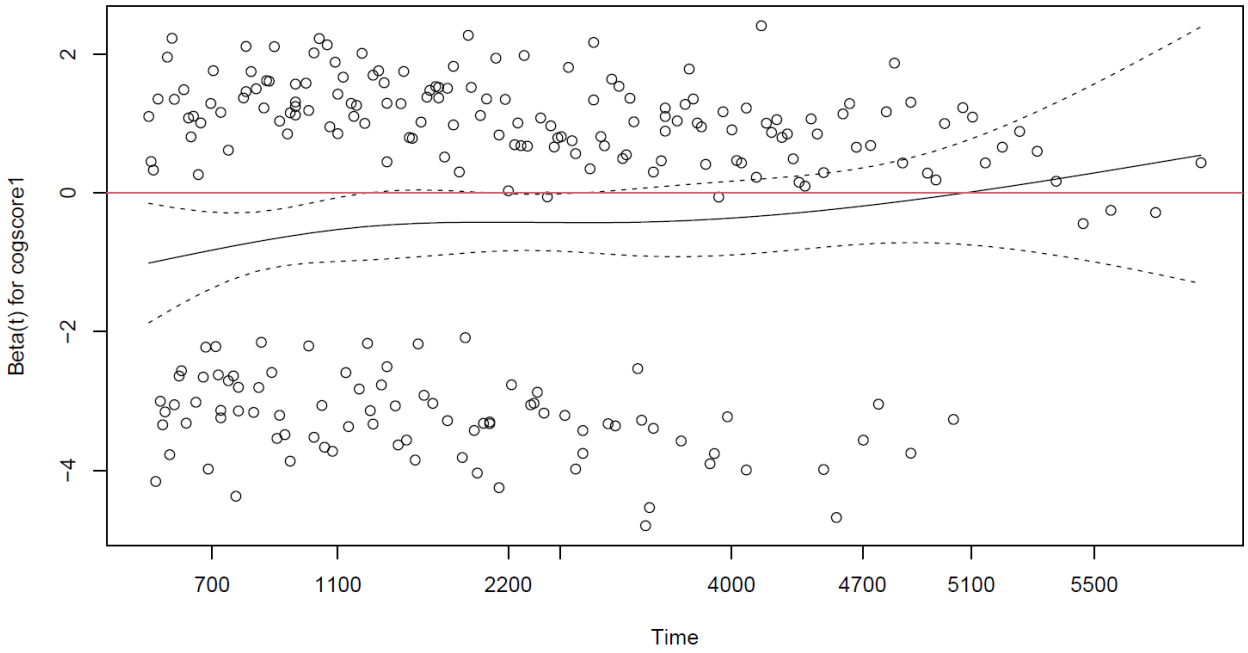
ln(θ) is the natural logarithm of the hazard ratio, or the log-hazard ratio

In the REGARDS cohort, a total of 946 (44%) had elevated CRP levels with concentrations exceeding 3 mg/L. A total of 310 participants (13.6%) developed dementia over the course of follow-up. Given these exposure proportions and the rate of dementia, a total sample size of N = 2,529 is required to observe a 20% increase in hazard at 80% power and a level of significance of 0.05.

In the CHS cohort, a total of 1,286 (38%) had elevated CRP levels with concentrations exceeding 3 mg/L. A total of 480 participants (14%) developed dementia over the course of follow-up. Given these exposure proportions and the rate of dementia, a total sample size of N = 2,570 is required to observe a 20% increase in hazard at 80% power and a level of significance of 0.05.

Appendix C. Schoenfeld Plot

Figure C-1. Scaled Schoenfeld residuals of baseline SIS score versus time from the REGARDS study



Appendix D. Martingale Plots

Figure D-1. Plot of the Martingale residuals for dementia versus age in the REGARDS study

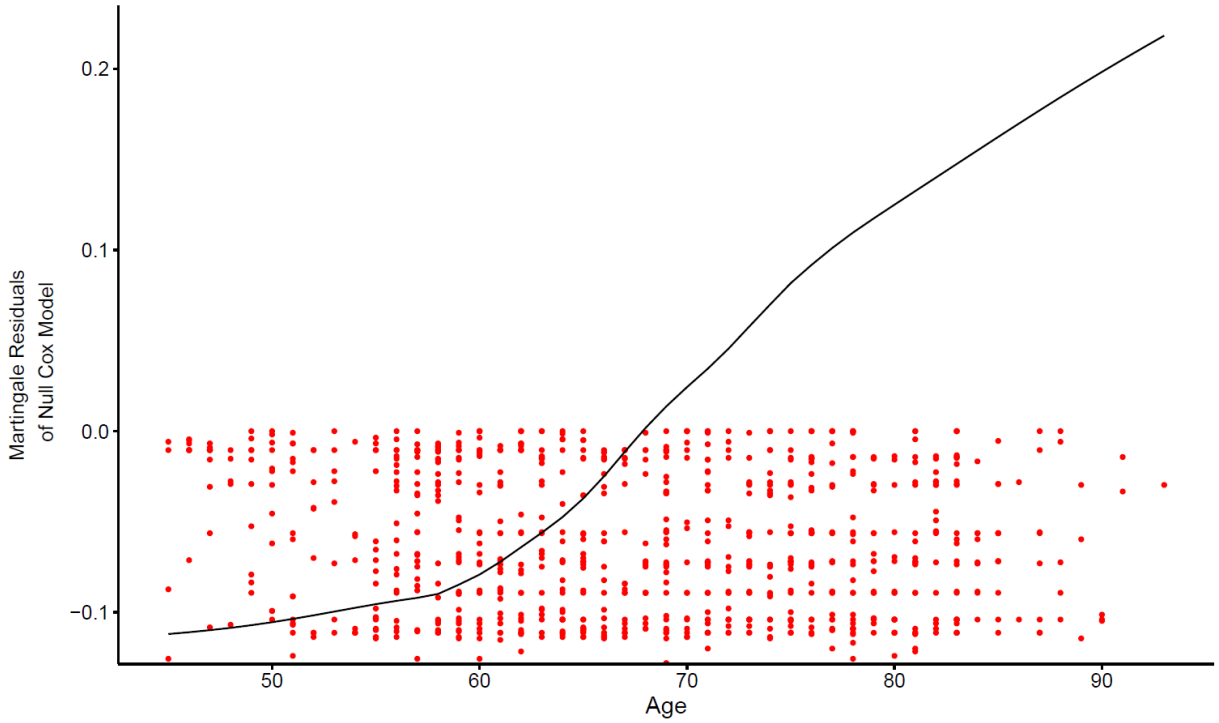


Figure D-2. Plot of the Martingale residuals for death versus age in the REGARDS study

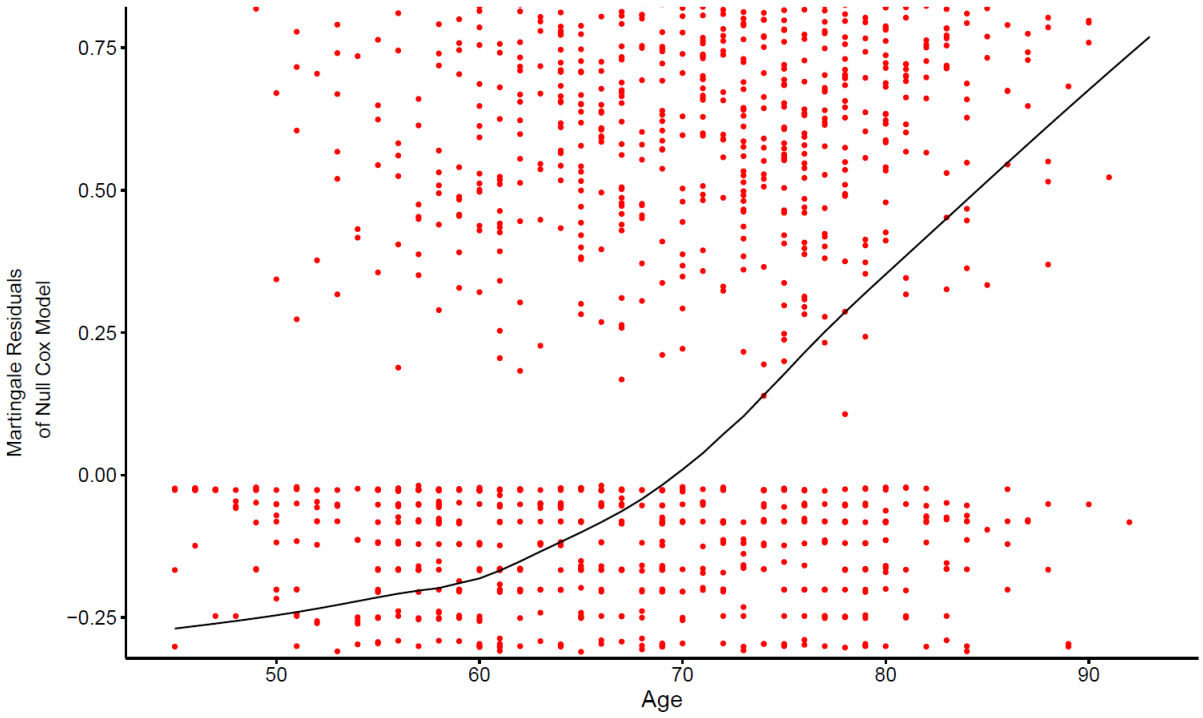


Figure D-3. Plot of the Martingale residuals for dementia versus age in the CHS cohort

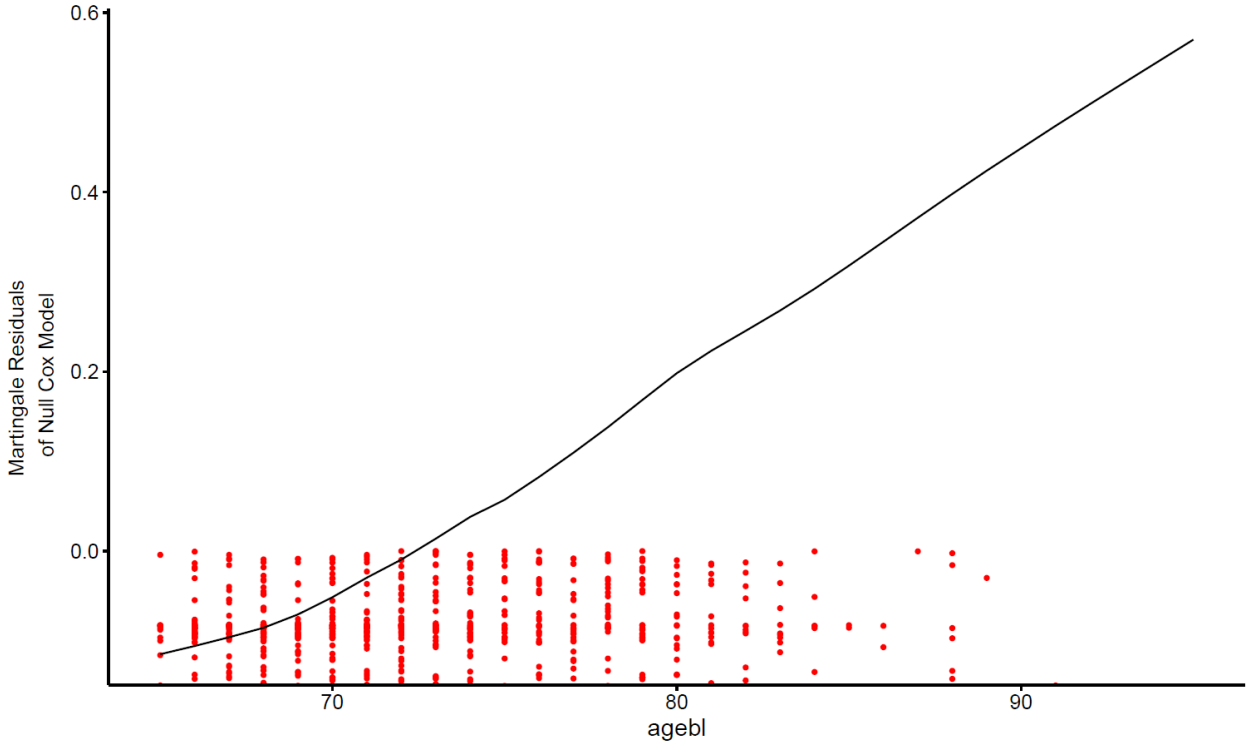


Figure D-4. Plot of the Martingale residuals for death versus age in the CHS cohort