Systemic Inflammation, Mild Cognitive Impairment and Alzheimer's disease: Findings from the PREVENT study.

by

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BScH (Biology), Queen's University, 2005
MSc (Biology), Lakehead University, 2008

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Abstract

The search for reliable early indicators of age-related cognitive decline represents an important avenue in aging research. Most research on late-life development charts cognitive change as a function of chronological age (CA), however, although CA is a commonly used developmental index, it offers little insight into the mechanisms underlying cognitive decline. In contrast, biological age (BioAge), reflecting the vitality of essential biological processes, represents a promising operationalization of developmental time. My overall programmatic doctoral research interests involve the identification of biological risk factors that predict age-related cognitive decline, impairment and dementia. In this dissertation document, I present: an overview of my empirical contributions to the BioAge and cognitive aging literature throughout my doctoral training; the dissertation project which uses preliminary data from the PREVENT study and provides evidence that elevated plasma pro-inflammatory proteins are associated with cognitive status (healthy controls (HC) vs Alzheimer's disease dementia (AD)), cognitive performance and are related to poorer cognitive performance in amnestic mild cognitive impairment (a-MCI); and a discussion on the broad implications of the project results and future directions in BioAge research.

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Dedication

Meo amare parentes

Chapter 1: Preface

Most research on late-life development charts cognitive change as a function of chronological age (CA). As described previously [1], CA as an index of developmental time carries no causal implications and little explanatory power. CA represents a dimension along which causal processes operate [2,3], reflecting the accumulation of biological and environmental influences contributing to developmental change [4,5]. The limitations of CA vis-à-vis informing mechanisms of cognitive change have been well argued [4–9]. Because it carries no causal association with developmental change, the utility of CA in isolation, may arguably even impede efforts to identify specific biological mechanisms underlying age-related cognitive impairment by placing a focus on a time metric rather than causal factors.

Advancing our understanding of the determinants and contributors of age-related cognitive decline requires that developmental time be operationalized using variables that index theoretical causes of cognitive aging [1,4,7]. Markers of biological health may more accurately indicate cognitive changes in late life compared to CA [8], as they likely reflect both the genetic make-up and environmental exposures that combine to influence cognitive function [10,11]. Targeting change in such potential mechanisms, rather than change in CA, may serve to strengthen the conceptual and practical framework of predictive models of change.

The concept of BioAge was originally developed because of the inaccuracy of CA as an index of development in old age [8]. BioAge has frequently appeared in scientific literature since its formal introduction by J.E. Birren in 1959, who initially defined it as the estimation of a person's present position with reference to their total potential lifespan. The BioAge concept has since expanded to reflect the functioning of essential physiological systems and processes in the body [4,8], to encompass a composite indicator of biological vitality (c.f. [1]), physiological

reserve [12], or a multivariate index of senescence [13], and to index the progressive age-related decrement of organism viability [14], or vigor [15]. Although the multitude of BioAge definitions and diverse methodology add to the controversy regarding its measurement, validity and usage [9,16,17], considerable progress in honing the concept and operationalizing candidate markers has been made (c.f. [1]) for both age-related health decrements [11,12,18] and cognitive decline [4,5,19–21]. Certainly, the utility of non-cognitive variables to predict differences in age-related cognitive performance has been a coveted focus for cognitive aging research (c.f. [6]), with an overarching goal to produce estimates that lead to an improved understanding of psychological aging and associated processes (c.f. [1]).

The majority of BioAge markers used to date can be classified as being indirect, or rather targeting more distal processes (e.g., health factors as well as physiological and sensory function) [1]. Although indirect measures such as physiological function (e.g., lung capacity, grip strength) are relatively accessible, inexpensive to measure and non-invasive, they also could be strong indicators of central nervous system (CNS) integrity and therefore may accurately predict age-related cognitive change [20]. Further, physiological functioning could indirectly index the functioning of underlying processes involved in cognitive functioning. For example, pulmonary capacity is likely related to physical activity levels, which have been associated with brain plasticity, brain-derived neurotrophic factor levels [22] and inflammation [23]; blood pressure can be a marker for hypertension, in which oxidative stress likely plays a key pathological role (c.f. [24]); and age-related declines in muscle strength can be indicative of sarcopenia, an age-related muscle degenerative disease, associated with high levels of inflammation and oxidative stress ([25]; c.f. [26]). Indeed, strong associations exist between physiological markers and cognitive functioning in older adults. Measures of sensory functioning, including visual and

auditory acuity, have been associated with age-related cognitive decline [4,27–31], whereas indicators of cerebrovascular dysfunction have been negatively associated with cognition [21,32,33]. Further, physiological functioning, such as weakened grip strength [34,35], lower limb strength [19], muscle strength [36] and reduced peak expiratory flow [37] have been linked to cognitive decline. Combining BioAge indicators, such as grip strength, health status, forced expiratory volume, vibration sense, hearing and vision demonstrated that BioAge markers can explain most age-related variance in cognitive performance [20,38] and can predict mortality in very old adults [29]. Further, a factor analytic approach, using biomarkers of visual and auditory acuity, grip strength, peak expiratory flow, blood pressure, and body mass index, predicted 12-year age-related cognitive decline in the elderly independent of CA [4]. Consistently, a BioAge index, computed using physical fitness measures (6 minute walk, weight lifting, balance, one-foot stand, standing reach, physical activity), cognitive performance, and disease comorbidity status predicted 3-year adverse outcomes better than using CA [12].

Despite the ability of indirect BioAge markers to account for age-related variance in cognitive performance, biological markers directly reflecting the functioning of key underlying processes will likely index cognitive function most accurately. It has been reasoned that more direct measures, which proximally target underlying biological processes theoretically relevant to cognitive decline and/or neurodegeneration should be employed in future research on cognitive aging [5]. Direct biomarkers symptomatic of the cellular processes involved in age-related cognitive change, such as vascular health, pro-inflammatory cytokines or oxidative stress by-products, could provide a more sensitive prediction of cognitive function while highlighting contributing processes [39]. Indeed, there has been a recent consensus that the incorporation of biomarkers indexing underlying disease states is an important future step to help improve

diagnostic accuracy of Alzheimer's disease dementia (AD) and preclinical disease phases [40,41].

In the field of cognitive aging, biomarkers of dementia have spanned a wide continuum of processes, ranging from genetic markers [42], peripheral blood proteins [43], CSF proteins [44] and brain beta-amyloid burden [45] to directly indexing brain atrophy [46]. Structurally indexing brain degeneration or beta amyloid accumulation (e.g., by using a PET amyloid ligand such as Pittsburgh compound B (PIB) [47], or [18F]FDDNP PET [48]) is informative in terms of understanding cognitive symptoms and trajectories. Similarly, elevated levels of CSF tau [44] and decreased levels of beta-amyloid 42 (c.f. [44]) have been found in AD patients and are associated with the future development of AD in patients with mild cognitive impairment (MCI) [49], with such proteins being increasingly used in clinical settings for disease detection and tracking. However, lumbar puncture poses substantial drawbacks in terms of expense and invasiveness, particularly in light of soaring dementia-related healthcare costs and frailty associated with the targeted demographic. Targeting the potential biological antecedents, such as inflammation [50,51], oxidative stress [52], neurovascular factors [53] or metabolic processes [54] that may underlie and/or exacerbate neurodegeneration, may be non-invasive ways to identify individuals at risk for future decline at the earliest stages. Evidence of a temporal ordering framework exists, whereby abnormal CSF biomarkers predate biomarkers of neurodegeneration and clinical symptoms, suggesting that biomarkers could identify changes prior to symptom onset [55]. To date, BioAge conceptualizations have largely overlooked the direct biological contributions to functional changes with age. In modern conceptualizations, recent scientific gains in biological research should be integrated to reflect critical pathways underlying cognitive change.

Alzheimer's disease, the most common cause of dementia, is an incurable, neurological disorder that is the sixth leading cause of death for adults ≥65 years in North America [56]. MCI is considered a high-risk intermediate condition for the progression to AD, whereby it is estimated that roughly 28.9% of individuals with MCI in population-based studies progress to AD, with higher annual conversion rates suggested for the a-MCI (11.7%) subtype compared to those with MCI with no memory impairment (4.1%) [57]. Although the diagnostic and statistical manual of mental disorders, 4th edition, text revision (DSM-IV-TR) has clear criteria for diagnosing dementia due to Alzheimer's disease pathology, the criteria for mild cognitive impairment classification remains variable [58–62]. Classification criteria for a-MCI have been adopted [63] to help further identify individuals who are at greater risk for Alzheimer's disease pathology (and progression to AD as a clinical outcome) given the higher annual conversion rates [57]. Recomendations have been introduced by the International Working Group on MCI to help clarify MCI criteria and to highlight additional information needed to further refine diagnostic criteria, including a better understanding of the etiology of MCI which could be aided by the identification of relevant biomarkers[62]. Relatedly, the international working group and recent theoretical persepectives have highlighted the important role of biomarkers for the diagnosis of AD [41], to enhance AD pathophysiological specificity [64], to help differentiate between Alzheimer's disease pathology and non-Alzheimer's disease pathology, to characterize the stage of cognitive impairment, and to help identify individuals with poorer clinical prognoses [65]. Even the role of biomarkers for the identification of presymptomatic individuals has become important with the introduction of the concept of preclinical Alzheimer's disease by the National Institute on Ageing, which highlights the role of biomarker evidence of AD pathology in clinically normal individuals ([66]). As beta amyloid levels in the brain may not always

correspond to the stage of brain neurodegeneration [65], further refinement and identification of mechanism-specific biomarkers linked to Alzheimer's disease pathology are needed.

Doctoral Programmatic Research

My programmatic doctoral research interests involve the identification of biological risk factors that predict age-related cognitive decline, MCI and AD. Improving BioAge models to incorporate mechanistic markers thought to be involved in the underlying neurological cascades of cognitive decline could accurately identify those at risk of AD and facilitate the early implementation of intervention strategies for modifiable risk factors. Throughout the past 5 years of study, I have contributed to many exciting research initiatives focused on investigating the association between biological risk factors and cognitive impairment.

First, using archival longitudinal data from the Victoria Longitudinal Study (VLS), I examined dynamic coupling between changes in theoretically relevant biological/physiological biomarkers (e.g., lung function) and changes in select cognitive measures. It was revealed that biological aging corresponded to declines in several cognitive outcomes, further supporting the notion that cognitive decline is not due to chronological age, but reflects causal factors (e.g., biological processes such as inflammation [67,68]) which accumulate along the age continuum (e.g., lifespan) [5]. Second, using archival data from Project MIND, a longitudinal investigation into the multidimensional factors influencing cognitive aging in healthy community-dwelling older adults, I found associations between several vascular risk factors (e.g., history of high blood pressure, heart disease and diabetes) and 8-year declines in cognitive and functional abilities (DeCarlo et al., 2010, unpublished findings). This research underscored the importance of vascular health in late life cognitive and functional performance even in healthy adults. Third, and relatedly, using longitudinal data from the VLS, I found time-varying covariation

associations between increases in various vascular risk factors (e.g., consumption of heart medications, reported high blood pressure) and up to 4-waves of cognitive decline in select cognitive outcome measures (e.g., fact recall, word recall) (DeCarlo et al., 2016, unpublished findings, manuscript in preparation). These findings are suggestive of a temporal relationship between the presence of within person vascular risk factors and cognitive functioning in the absence of significant cognitive impairment.

Fourth, genotyped older adults from the VLS were examined for independent and interactive associations of two genetic risk polymorphisms (APOE ε4 and COMT G/G) with respect to objectively classified cognitive status groups (MCI and healthy controls (HC)) at both baseline and across a 4-year longitudinal interval. The presence of at least one APOE ε4 allele was associated with an increased risk of baseline MCI status, and the presence of APOE ε4 and COMT G/G were associated with 4-year MCI chronicity (i.e., stable MCI over time) [69]; highlighting the importance of including genetic risk factors in predictive models of change. Fifth, as a follow-up, using cross-sectional and two-wave longitudinal data from the VLS, I examined separate and interactive associations between vascular (e.g., reported history of vascular diseases, measured lung capacity) and genetic risk factors (e.g., APOE ε4 and COMT G/G) with respect to MCI status at baseline or MCI conversion (converting from healthy to MCI) and MCI stability over a 4-year re-test interval. Indicators of vascular health separately and interactively with APOE ε4 were associated with risk of MCI at baseline and/or associated with MCI conversion or MCI stability over time [70]. These findings underscored the multi-factorial nature of cognitive impairment, and further highlighted the importance of vascular health in late life cognitive functioning and the importance of a stable MCI classification over time given the heterogeneous nature of the classification [71]. Sixth, extending previous research showcasing

the importance of vascular health on cognition, I recently explored the associations between vascular health risk factors and non-normative cognitive decline by investigating the links between vascular risk factors (e.g., reported history of TIA, reported history of high blood pressure, measured pulse pressure, reported physical activity levels) and both cognitive group status (e.g., MCI and mild/moderate AD) and cognitive performance in the PREVENT study, a cross-sectional, multi-factorial investigation into the risk factors for MCI and AD. The presence of vascular risk factors significantly differentiated between pathological and healthy groups (i.e., AD and MCI vs healthy controls (HC)). Moreover, results reveal that better vascular health was linked to better cognitive performance at baseline (DeCarlo et al., 2016, unpublished findings, manuscript in preparation); further substantiating the important role that vascular health plays in late-life cognitive functioning.

Collectively, these findings provide evidence that biological and physiological factors are associated with cognitive status and performance in late-life, whether it be age-related or non-normative cognitive decline. It has been suggested that multiple biological processes, such as inflammation and oxidative stress, should be the focus for the pathophysiological progression of late-life cognitive disorders, such as AD ([72]; c.f. [39]). Certainly, there is a surfeit of literature suggesting the important role that inflammation plays in the neuro-pathogenesis of AD, indicating that inflammatory mediators are a potential fruitful and theoretically relevant target for operationalizing BioAge to capture the underlying causes and early biological indicators of neurodegeneration (DeCarlo et al., 2014).

Chapter 2: Dissertation Project

Abstract

Background. Amnestic mild cognitive impairment (a-MCI) is a high-risk condition for progression to Alzheimer's dementia (AD). Inflammation is a key mechanism underlying neurodegeneration. **Objective.** We examined whether there were significant differences in peripheral inflammatory protein levels amongst cognitive status groups (healthy controls (HC), a-MCI, AD) or whether inflammatory protein levels were associated with neuropsychological performance across and within cognitive status groups. **Methods.** We used cross-sectional data from 49 participants (Age = 74.02 (5.73); Education = 14.70 (3.39); Gender = 59.2% Women) enrolled in the PREVENT study, a cross-sectional, multi-factorial investigation of risk factors for a-MCI and AD. Data included plasma protein levels of Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α), Macrophage Migration Inhibitory Factor (MIF) and Monokine Induced by Gamma Interferon (MIG) analyzed on the Bio-Plex multiplex system, as well as cognitive data from a wide variety of neuropsychological tests. **Results.** Using analyses of covariance, MIG protein levels differed significantly between HC and AD groups. Regression analysis revealed that higher TNF-α and MIG protein levels were significantly associated with poorer 3MS performance, irrespective of cognitive status. Higher IL-6, TNF-α and MIG protein levels were significantly associated with poorer 3MS and Rey Auditory Verbal Learning Task long delayed memory trial (RAVLT A7) performance in the a-MCI group. **Conclusion.** This study adopted a novel approach whereby a unique subset of plasma pro-inflammatory proteins were analyzed in the context of a comprehensive neuropsychological battery and clinical classification of a-MCI and AD. These results provide evidence that there are group differences in plasma pro-

inflammatory protein levels (HC vs AD) and that elevated pro-inflammatory protein levels are related to worse cognitive performance, particularly in a-MCI.

Introduction

Inflammatory processes are a major contributing factor in many age-related diseases and it is thought that better understanding and management of the interactions between the immune system and the central nervous system may be critical in preventing or delaying many neurological conditions (c.f. [73]). Certainly, there is mounting evidence of the role of inflammation in the pathogenesis of Alzheimer's dementia (AD), particularly in early disease phases [74]. Although there is some evidence that a pro-inflammatory process may be initiated prior to plaque formation (c.f. [75]), it has been demonstrated that beta amyloid induces the expression of pro-inflammatory cytokines in cultured microglia [76]. Aberrant aggregates of beta amyloid likely trigger immune system activation as a host response against an invading pathogen. Pattern recognition receptors stimulate an innate immune response that, in an effort to clear beta amyloid aggregates, leads to neuronal dysfunction, neurodegeneration [77] and, over time, chronic neuro-inflammation in the face of sustained levels of aggregated beta amyloid that cannot be cleared [78]. Indeed, faulty resolution of the inflammatory response has been demonstrated in the hippocampus of individuals with AD [79].

Pro-inflammatory cytokines and chemokines produced by microglia in the brain could conceivably influence systemic pro-inflammatory protein levels by their release into the blood [80], and vice-verse, peripheral cytokines can cross the blood-brain barrier to influence the CNS inflammatory milieu [81]. Indeed, peripheral inflammation can exact changes in pro-inflammatory cytokine levels within sensitive brain regions, like the medial temporal lobe (c.f. [82]). Correspondingly, serum IL-6 levels correlated with CSF levels taken from subjects with

probable AD [83]. Elevated levels of systemic pro-inflammatory cytokines, such as interleukin-6 (IL-6) [84,85], Interleukin-1 [84], Interleukin-18 (IL-18) [86] and tumor necrosis factor alpha (TNF-α) [84,85,87] as well as the pro-inflammatory macrophage migration inhibitory factor (MIF) and monokine induced by gamma interferon (MIG) [88] have been found to be elevated in the blood of AD patients. Similarly, in individuals with MCI, elevated blood levels of TNF-α [89], MIF [88], IL-6 [68], IL-18 [86] and interleukin-8 (IL-8) [90] have been found.

IL-6 and TNF-α are pro-inflammatory cytokines that are secreted by multiple cells of the immune system in response to pathogen invasion or injury. In the brain, they are produced by activated microglia in response to stressors such as beta amyloid, as well as aberrant Nuclear Factor Kappa B (NF-κB)-related responses in neurons triggered by accumulated phosphorylated tau and beta amyloid and the presence of pro-inflammatory cytokines themselves (c.f. [50]). MIF is a pro-inflammatory lymphokine that supports immune system function and has been shown to bind directly to beta amyloid [91] and stimulate macrophages to produce TNF. MIG is a cytokine that plays a role in regulating CNS inflammatory responses [92] and is directly activated by interferon gamma (IFN-γ), a type 2 interferon involved in innate immunity. Although IL-6, TNF-α, MIF and MIG are up-regulated (i.e., gene activation) by different mechanisms, IL-6, TNF-α and IFN-γ are tightly tied to NF-κB nuclear translocation in innate immune responses, suggesting that MIG (induced by IFN-γ), IL-6, and TNF-α expression levels in particular may be closely related. To the extent that systemic pro-inflammatory mediators may reflect enhanced neuro-inflammatory cascades [80], measuring levels of these proteins could represent a sensitive and relatively non-invasive approach to identifying those at risk for a-MCI and AD [39,85,89].

The pathology of cognitive decline likely begins years prior to detection of clinical symptoms, with detection usually coinciding with irreversible neuronal damage. Identifying

mechanism-related risk factors for a-MCI and AD may not only improve the theoretical understanding of the biological processes underlying AD and its prodrome, but it may also increase the accuracy of predictive methods (cf. [39]) because of the temporal ordering of aberrant biomarkers [55]. Certainly, there have been a multitude of research initiatives that have identified biomarkers linked to increased risk of MCI conversion to AD such as CSF T-tau [93], plasma cytokines [94], and blood protein markers [85,95]. However, investigations employing multiple blood proteins indexing a unifying cellular network (e.g., inflammation) are infrequent. Multi-modal approaches utilizing a combination of biological markers (i.e., CSF tau/beta amyloid, glucose metabolism, APOE status), MRI techniques and/or neuropsychological testing to predict MCI conversion to AD have recently surfaced in the literature [93,94,96–99], with prediction accuracy estimates ranging from 68.5% to 97%. There continues to be a need for the augmentation of current predictive models with biological indicators that a) are sensitive to the theoretically relevant mechanisms involved in the pathological process, b) can be detected prior to the onset of clinical symptoms), c) can inform future intervention strategies to potentially delay symptom onset, and d) can be measured through quick and minimally invasive methods, for ubiquitous and cost-effective detection of individuals at risk for MCI and AD.

Although extant literature has identified a link between peripheral inflammatory processes and AD [83–88] and MCI [68,86,88–90], to our knowledge this unique battery of systemic pro-inflammatory proteins, consisting of IL-6, TNF-a, MIG and MIF, has not been widely examined with respect to a-MCI, AD or comprehensive neuropsychological examination across and within cognitive status groups. Our objective is to extend previous findings and understand the link between systemic inflammatory protein levels, cognitive status and cognitive performance in the context of individuals classified with a-MCI or AD compared to healthy

controls (HC) to highlight mechanistically-relevant biological risk factors that could augment current strategies for identifying those at risk for cognitive impairment and AD. Although biological risk factors can certainly be useful to identify those at risk of future cognitive impairment or dementia (i.e., prognostic modelling), we examine risk factors linked to diagnostic classifications in this study. We investigated two main research questions towards this objective (Table 1), using planned comparisons. First, the associations of measured plasma protein levels of MIF, MIG, IL-6 and TNF-α with cognitive group status were examined (i.e., HC, a-MCI, and AD), and in particular we examined whether there were specific group differences in inflammatory protein levels in a-MCI and AD compared to HC, respectively. Second, the associations of measured plasma protein levels of MIF, MIG, IL-6 and TNF-α with cognitive performance were examined, with particular emphasis on associations across all individuals as well as separately in the HC and a-MCI groups. It was hypothesized that higher levels of inflammatory proteins would be linked to a-MCI and AD status compared to the HC group, in line with previous findings [88]. It was also hypothesized that higher levels of pro-inflammatory proteins would be differentially associated with cognitive domains and correspond to brain areas associated with neuropathological changes typically found in individuals with a-MCI and early stages of AD, such as visual and verbal memory deficits associated with altered functioning of the medial temporal lobe.

Method

Participants

This research was conducted under full human ethics approval by both the Human Research Ethics Board at the University of Victoria and the Vancouver Island Health Authority in keeping with the Canadian Tri-Council Policy Statement: Ethical Conduct for Research

Involving Humans. Written informed consent was obtained from all participants. Participants were community-dwelling older adults from the Victoria area participating in the PREVENT study, recruited through advertisements in the public media and to community groups. The PREVENT study is a cross-sectional, multi-factorial (e.g., biological, physiological, environmental) investigation of risk factors for MCI and AD involving individuals who are cognitively healthy, or who meet classification criteria for a-MCI or probable mild/moderate AD. Exclusionary criteria for participant enrollment included a newly diagnosed psychiatric disturbance within the past year (i.e., Major Depressive Disorder), history of a chronic neurological condition (i.e., Parkinson's disease, brain tumor), serious episodes of cardio/cerebrovascular disease (i.e., heart attack, stroke, heart surgery) within the past year or other factors that could contribute to changes in cognitive functioning (i.e., head injury, vitamin deficiency), factors that could directly result in cognitive deficits or impairment not reflective of emerging neurodegenerative conditions consistent with AD or its prodrome. Severe sensory/motor impairment (i.e., unable to read newspaper-sized print with glasses, difficulty writing or pressing keys on a keyboard, or unable to hear a normal spoken conversation adequately with the use of a hearing aid) precluded enrollment in the study. Classification procedures are described in detail below.

Community-dwelling individuals over the age of 65 were recruited for the study, so that late onset pathology was targeted. Of the individuals whose pro-inflammatory protein levels were in the detectable range for all 4 proteins analyzed (49/52), n= 23 participants were classified as HC (Age: M = 73.17, S.D. = 5.83; Gender: 73.9% women; years of education: M = 14.89, S.D. = 2.76), n=20 met criteria for the a-MCI group (Age: M = 73.80, S.D. = 5.26; Gender: 50% women; years of education: M = 15.15, S.D. = 4.11) and n=6 met criteria for the

AD group (Age: M = 77.43, S.D. = 5.86; Gender: 33.3% women; years of education: M = 13.29, S.D. = 5.86). Individuals who did not have detectable protein levels for all 4 inflammatory proteins (n=3) were not included in the analyses.

Cognitive Status Classification

To classify individuals as belonging to HC, a-MCI, or AD groups, we used a standard and objective classification system that involved both neuropsychological test scores and clinical judgement, consistent DSM-IV-TR criteria ([100]) (see Appendix A for the PREVENT study classification profile sheet). The procedure emphasized objective assessments of performance in select neuropsychological domains, such as memory, executive function and language. Two advanced doctoral students in clinical neuropsychology classified individuals independently, with author H.T. making the final classification decision based on a full evaluation of the participant file when student classifications differed (n=2), to ensure standard classification procedures. The full cognitive battery and normative samples used are described below.

To meet criteria for the cognitively healthy group, participants were required to perform > than -1.0 S.D. on all cognitive domains in the absence of self-reported memory complaints or reported impairment in social, occupational or daily life functioning and no active neurological disease. To meet criteria for the a-MCI group, we employed a conservative approach, that deviated slightly from previously instituted criteria where a cut-off score of ≤ -1.5 SD was used ([101,63,58,62]). In the current study, in order to capture early cognitive changes, non-demented participants with self-reported or informant-reported memory complaints were required to perform ≤ to -1.0 S.D. below the mean on at least one memory task and perform > than -1.0 S.D. on all other cognitive domains, in the absence of active neurological disease or reported impairment in social, occupational or daily life functioning. Although some recently employed

MCI definitions rely on objective ways to measure intact activities of daily living (e.g., Clinical Dementia Rating Scale = 0.5) or generally preserved cognitive functioning (e.g., MMSE ≥ 24) [59,60], our classification method emphasizes clinical judgement based on participant and informant report.

To meet criteria for the AD group, following DSM-IV-TR guidelines for dementia of the Alzheimer's type, participants were required to perform ≤ -2.0 S.D. in two cognitive domains, one of which was memory, with the presence of subjective or collateral-reported significant declines from previous levels of functioning in both domains with deficits resulting in reported impairment in social, occupational or daily life functioning. The onset of impairment must have been gradual and the course progressive. Self-report or collateral-report of information pertaining to the participants social, occupational, or daily life functioning was obtained through a structured interview with the participant and/or their family member/close friend either by phone or in person (see collateral interview in Appendix A).

Neuropsychological Battery

The neuropsychological battery included measures that represent the following cognitive domains, as outlined in Table 2; global cognitive functioning (Modified Mini-Mental State Test (3MS)), auditory attention (WAIS-R Digit Span (Total score)), auditory working memory (WAIS-R Digit Span Backwards), visual memory (Benton Visual Retention Task, multiple choice), auditory immediate and delayed memory (Rey Auditory Verbal Learning Task (RAVLT); Total, A6 (short delay), and A7 (long delay)), executive functioning (WAIS-R Similarities (short-form) and Trails B), language (Controlled Oral Word Associations Test (CFL) and Animal Naming), visuo-construction (WAIS-R Block Design (short-form)), processing speed (Trails A and WAIS-R Digit Symbol). Scoring procedures used in the Canadian Study of

Health and Aging (CSHA;[102]) were implemented for the WAIS-R short-form subtests. Normative data from the CSHA was used for calculating age, gender and education based performance on all WAIS-R subtests, the RAVLT interference (A6) and long-delayed (20 minutes) (A7) trials, BVRT, Controlled Oral Word Associations Test (norms for FAS were used) and Animal Naming tests. Normative data derived from Mayo's Older Americans Normative Studies were used for calculating age-based performance on Trails A, Trails B and the delayed recall trial of the RAVLT due to the lack of availability of normative data for these tasks in the CSHA. Individuals over the age of 90 (n=1; age 93, MCI group) were compared to 90 year olds in the CSHA reference sample due to the availability to normative information.

Peripheral Inflammatory Markers

Whole blood samples were taken from participants after an overnight fast. Blood was collected by a certified Phlebotomist at the Deeley Research Centre (Victoria, British Columbia) between 7am and 11am on a day convenient to the participant within 6 months after completing the health questionnaires and the cognitive and physiological assessment portions of the study. Plasma was isolated from whole blood specimens within 60 minutes of the blood draw according to a standard Ficoll gradient procedure. Plasma samples were stored in 2ml aliquots at -80°C until analyzed. We measured IL-6, TNF-α, MIF and MIG protein levels using the Biorad Bio-Plex multiplex system with cytokine assays (Human Cytokines Group 1, 2-plex (IL-6, TNF-α); Human Cytokines Group 2, 2-plex (MIG, MIF), Biorad, Veenendaal, The Netherlands), according to the manufacturers' instructions. Samples were analyzed in undiluted form due to concerns over low expression levels for IL-6 and TNF-α. The detection ranges were 2.3-18,880 pg/mL, 0.2-2,059 pg/mL, 231-24,373 pg/mL and 2.7-8,584 pg/mL for IL-6, TNF-α, MIF and

MIG, respectively. Of the 52 samples analyzed, 49 samples yielded detectable levels for all 4 proteins and hence were used in the statistical analyses.

Anti-Inflammatory Medication Use

Current self-reported use of anti-inflammatory medication was controlled for in all analyses. Individuals were assigned a value of "1" if they reported currently using any type of medication that was anti-inflammatory in nature, over and above 81mg of aspirin daily (compared to 0 = no anti-inflammatory medication use or only daily consumption of 81mg of aspirin), similar to other approaches to categorizing NSAID use [103].

Data Analysis

All analyses were performed using SPSS Version 18 (IBM SPSS Statistics). Statistical significance was determined using $p \leq 0.05$. Gender and current anti-inflammatory medication consumption were entered as covariates in all analyses separately (due to sample size constraints), as both gender [104–106] and anti-inflammatory medication can influence systemic pro-inflammatory protein levels. Further, although other factors could influence systemic inflammation levels, such as the presence of health conditions linked to heightened levels of inflammation (e.g., cancer, rheumatoid arthritis, fibromyalgia, chronic pain), the heterogeneous nature of such conditions and the inter-individual differences within each condition made it difficult and arguably uninformative to combine them under one unifying "pro-inflammatory" condition group for covariation purposes. Further, although some studies have controlled for such factors (i.e., the presence of rheumatoid arthritis [107]), this approach would not capture whether or not individuals consume anti-inflammatory medications to help manage such conditions. Therefore, it was rationalized that indexing current anti-inflammatory medication use, independent of health status, was an optimal way to control for non-pathological

contributions that could influence pro-inflammatory protein levels in isolated plasma. For research question #1, a series of analyses of covariance were performed to examine differences in pro-inflammatory protein levels between the three cognitive status groups. For research question #2, regression analyses were performed to examine whether pro-inflammatory proteins were associated with cognitive performance on select neuropsychological tasks both irrespective of and within cognitive status groupings (i.e., HC and a-MCI). As some cognitive tasks were used for classification as well as outcome variables (e.g., RAVLT, Trails B, WAIS-R Block Design), analyses were performed across and within cognitive status groups, with the latter approach serving to minimize circularity bias (i.e., any observed association with individual differences in cognitive function within cognitive status groups would not be confounded with classification status). Apart from analyzing associations with raw protein levels, inflammatory protein levels were also dichotomized into "high" vs "low" levels based on their frequency distribution (i.e., 50^{th} percentile) and associations with cognitive performance in all domains across and within cognitive status groups (i.e., research question 2a and 2b) were examined. This was undertaken as a first approach to understanding the utility of employing cut-off scores with respect to plasma pro-inflammatory protein levels. Due to the modest sample size of the AD group, analysis of cognitive performance using raw protein levels or dichotomized protein groupings within the AD group was not performed due to insufficient power. Given our *a priori* hypotheses regarding expected directional effects, we employed one-tailed tests for all analyses.

Results

Participant demographic characteristics and comparisons are reported in Table 3. We computed planned and inferential tests of between-group differences with respect to the demographic variables. Age, gender and educational attainment differed between the HC and AD

groups. In the absence of significant correlations between inflammatory protein levels and age and educational attainment, only gender was controlled in the analyses to account for potential gender differences in peripheral cytokine levels across the lifespan [105]. We report results pertaining to the associations between inflammation and cognitive status and performance.

Inflammation and Cognitive Status

Analyses of covariance were used to examine whether mean protein levels differ as a function of cognitive status. As shown in table 4, MIG protein levels differed significantly (p ranged from 0.05 – 0.05; 1-tailed) between the HC and AD groups, with higher levels found in the AD group, even after gender and anti-inflammatory medication use were controlled. MIF levels differed significantly between the HC and AD groups when gender was controlled but not on its own or when the use of anti-inflammatory medication use was controlled for) (p = 0.05; 1-tailed), with higher levels found in the AD group. Notable non-significant trends also exist for TNF-α protein levels between the HC and AD groups (p ranged from 0.08 – 0.08 (TNF-α), with higher levels found in the AD group.

Inflammation and Cognitive Performance

Associations Across Cognitive Status Groups

Regression analyses were performed to examine the predictive capacity of pro-inflammatory protein levels for cognitive performance across a wide range of cognitive tasks, irrespective of cognitive status. As shown in table 5, higher TNF-α levels were significantly associated with poorer performance on the 3MS, even after gender and anti-inflammatory medication use were controlled (p ranged from 0.03 – 0.03; 1-tailed), where a 100 pg/mL increase in TNF-α levels was associated with a 2.3-2.4 total score decrease in 3MS performance. Similarly, higher MIG levels were also significantly associated with poorer performance on the

3MS, even after gender and anti-inflammatory medication use were controlled for (p ranged from 0.03 – 0.04; 1-tailed), where a 100 pg/mL increase in MIG levels was associated with a 0.5 total score decrease in 3MS performance.

In terms of delayed verbal memory, higher TNF-α levels were significantly associated with poorer performance on the RAVLT A7 trial, even after gender (but not anti-inflammatory medication use) was controlled for (p ranged from 0.05 – 0.05; 1-tailed), where a 100 pg/mL increase in TNF-α levels was associated with a 0.8-total score decrease in RAVLT A7 performance. Notable non-significant trends also existed for IL-6 and MIG protein levels, whereby higher protein levels were associated with poorer performance on the 3MS and RAVLT A7, respectively (p ranged from 0.07 – 0.08 (IL-6) and 0.07 – 0.08 (MIG); 1-tailed).

Simple Effects within Cognitive Status Groups

Regression analyses were also performed to examine the predictive capacity of pro-inflammatory protein levels for cognitive performance across a wide range of cognitive tasks separately within the HC and a-MCI groups. As shown in table 6, higher IL-6, TNF-α and MIG levels were significantly associated with poorer performance on the 3MS in the a-MCI group, even after gender and anti-inflammatory medication use were controlled. In particular, a 100 pg/mL increase in IL-6, TNF-α and MIG levels was associated with a 9 (p ranged from .03 - .05; 1-tailed), 2 (p = .01; 1-tailed) and 1 (p ranged from .02 - .04; 1-tailed) respective unit decrease in the total 3MS score in individuals with a-MCI. Additionally, higher IL-6, TNF-α and MIG levels were significantly associated with poorer performance on the Block Design task in the a-MCI group. However, the significant associations with Block Design were attenuated once gender and anti-inflammatory medication use were controlled. Finally, after dichotomizing protein levels into “high” vs “low” levels based on their frequency distribution within the a-MCI group, higher

IL-6, TNF-α and MIG levels were significantly associated with poorer performance on RAVLT_A7 in the a-MCI group, even after gender and anti-inflammatory medication use were controlled. In particular, individuals having IL-6, TNF-α or MIG levels higher than the 50th percentiles for each protein (i.e., higher than 6.84, 20.77, and 195.78 pg/mL for IL-6, TNF-α and MIG, respectively) had a 2.4 – 2.7 (p = ranged from 0.02 to 0.03; 1-tailed), 2.8 – 2.9 (p = ranged from 0.01 to 0.02; 1-tailed) or 3.0 – 3.1 (p = 0.01; 1-tailed) point lower total score in RAVLT_A7 performance compared to individuals with protein levels below the 50th percentile in the a-MCI group. No other significant associations were found with respect to cognitive performance in the HC or a-MCI groups.

Discussion

Identifying mechanism-related risk factors for a-MCI and AD may increase the accuracy of future predictive models and lead to improved theoretical understanding of the biological mechanisms driving cognitive decline and AD (cf. [54,96]). (cf. [5,39]). Although definite disease-modifying therapies are not currently available, identifying underlying risk factors could facilitate prediction of individuals at future risk for cognitive impairment and AD, inform potential preventive strategies that could delay or arrest neurodegeneration ([108]) (i.e., by targeting biological mechanisms, such as inflammation [109,110], or overall physical health [109,111]) or increase compensation strategies/enhance memory self-efficacy[112], or, at the very least, provide additional time to plan and prepare for the future. Due to the link between aberrant neuro-inflammatory cascades and AD [113,114], the likely association between peripheral- and neuro-inflammation, as well as the relatively non-invasive methods required to measure plasma inflammatory proteins, indexing systemic inflammation represents a promising enterprise in this regard. Using cross-sectional data, we examined whether mean plasma protein

levels of IL-6, TNFα, MIF and MIG differed as a function of cognitive status (i.e., a-MCI or AD group versus HC), or were associated with neuropsychological performance irrespective of or within cognitive status groups. This investigation substantiates previous findings linking peripheral inflammation in MCI and AD and extends this literature by encompassing many novel additions, such as; a) investigating a unique battery of 4 systemic pro-inflammatory proteins that not only indexes a unifying cellular network but has not been widely examined with respect to mild/moderate AD or, in particular, individuals with a-MCI, b) including clinical classification of a-MCI and mild/moderate AD, c) examining associations between peripheral inflammation and comprehensive neuropsychological performance in cognitively healthy individuals and those with a-MCI exhibiting early cognitive changes, d) utilizing methodology (Biorad Bio-Plex) sensitive enough to detect low expressing plasma proteins, such as IL-6, e) highlighting a biological process that is not only detectable through non-invasive methodology but has potential for future intervention strategies to help delay or prevent symptom onset, and f) controlling for covariates that have been shown to influence peripheral inflammation, such as gender and anti-inflammatory medication use; the latter of which is performed very infrequently in the literature.

First, we identified significant mean differences in peripheral inflammatory protein levels amongst cognitive status groups. Our analyses revealed that MIG protein levels were different between the HC and AD groups, with higher levels found in the AD group. These results are consistent with previous findings where MIG was shown to be elevated in AD patients compared to individuals who were not cognitively impaired [88,115]. Further, consistent with previous findings [88], MIF levels differed significantly between the HC and AD groups, with higher levels found in the AD group when gender differences were controlled. However, when anti-inflammatory medication consumption was entered as a covariate, elevations in MIF levels in the

AD group diminished indicating that MIF may be influenced by exogenous factors and that failing to control for anti-inflammatory consumption could possibly result in spurious associations between MIF and cognitive status. Although trends were found for TNF-α differentiating AD from HC, coinciding with previous findings [84,85], IL-6 failed to show any association with cognitive status, a result which mirrors contradictory findings in the broader IL-6 literature, possibly owing to a great degree of inter-individual variances in IL-6 levels within and across studies [116]. Finally, contrary to previous findings linking higher peripheral inflammation to a-MCI [68,117], we did not find significant group associations between inflammatory protein levels and classification of a-MCI compared to the HC group. This may partially reflect the conservative (i.e., -1.0 S.D.) a-MCI classification procedures used which aimed to target early stages of cognitive impairment but may have captured individuals undergoing transient or non-neuropathological age-related cognitive changes. Relatedly, the instability of MCI status has been well documented, with close to 30% of those with baseline MCI status reverting to no cognitive impairment at 2 to 3 year follow-up [118,119].

Second, we examined the association between peripheral inflammatory mediators and neuropsychological function, an area that has been largely overlooked in the literature. Our findings indicate that higher pro-inflammatory protein levels are associated with poorer performance on certain cognitive tasks regardless of cognitive status. Higher TNF-α and MIG levels were associated with poorer overall performance on the 3MS. As poorer cognitive performance tends to be associated with progression to dementia in individuals with MCI [99], the link between inflammation, a theorized pathogenic process involved in AD, and 3MS performance in the current study was foreseeable. Although significant associations were not found between IL-6 levels and MMSE performance, a trend existed whereby higher IL-6 levels

were linked to poorer 3MS performance, coinciding with previous findings linking higher IL-6 levels with poorer performance on the MMSE and Montreal Cognitive Assessment [67,68].With regard to delayed memory, higher TNF-α levels were associated with poorer performance on verbal long delayed memory, after gender differences were controlled for. This result is consistent with previous reports indicating that a latent cellular inflammation factor, consisting of, among others, IL-6 and TNF-α, was associated with poor memory performance in non-demented older adults [106]. Further, peripheral inflammation has been associated with reduced spatial memory performance in young males in the absence of cognitive impairment [82]. However, controlling for consumption of anti-inflammatory medications diminished the association between TNF-α levels and delayed memory performance, indicating that anti-inflammatory medication use may influence peripheral TNF-α protein expression and not controlling for anti-inflammatory medication use could artificially strengthen TNF-α associations. Finally, unlike IL-6, TNF-α and MIG, MIF did not show any associations or trends with cognitive outcome measures across or within cognitive status groups in the current study, which could reflect disparate activation pathways for MIF in innate immune responses.

Third, despite finding no significant group differences in inflammatory protein levels, our analyses revealed that pro-inflammatory protein levels are differentially associated with cognitive performance on certain tasks within the a-MCI group, but not in the HC group. Individuals classified with a-MCI with higher levels of IL-6, TNF-α and MIG plasma proteins performed significantly worse on the 3MS and the RAVLT delayed episodic memory task, corresponding to a similar study which demonstrated a negative association between serum IL-6 levels and both MMSE and long-delayed episodic memory performance in a-MCI [68]. The patterns shown with respect to inflammatory protein levels and Block Design performance are

tenuous and indicate that gender differences and anti-inflammatory medication use can considerably influence these associations. Interestingly, IL-6, TNF-α and MIG, which are upregulated via similar cellular pathways, all show similar qualitative associations with the same cognitive outcomes, perhaps highlighting a unifying signalling network. Further, the magnitude of the negative association between IL-6 and 3MS performance in comparison with the other proteins is noteworthy and, to our knowledge, has not been reported to date. Although the mechanism underlying this difference in magnitude is poorly understood and relevant literature is sparse, it is hypothesized that because IL-6 persists in the plasma much longer than other pro-inflammatory cytokines (c.f. [120]), this persistence could lead to more widespread cognitive impact than other cytokines. Identification of the sub-components of the 3MS that are most strongly negatively associated with peripheral inflammation levels could be the subject of future research. Overall, these findings suggest that among those with mild memory impairment in the absence of functional impairment, peripheral inflammation seems to be related to not only worse memory performance but worse global cognitive functioning as well, which is consistent with available literature indicating that inflammatory markers may be useful in predicting MCI progression to AD [94].

Several limitations should be acknowledged. First, although the current sample size was consistent with previous studies, statistical power was considered assuming a moderate effect size, and many associations were documented in line with our hypotheses, overall power was limited, particularly with respect to the AD group. Sample size limitations also resulted in large confidence intervals in AD group analyses and limited our ability to include multiple covariates within models. Future research should aim to increase the sample size to facilitate more stringent tests, however, notwithstanding the small sample size, the identified patterns were consistent

with theoretical expectations. Second, although significant strides have been made towards the classification of MCI [62], a global consensus has not been reached as to its precise operationalization. Utilizing a conservative -1.0 S.D. cut-off approach to identify early changes may have captured normative age-related changes or those likely to revert to no cognitive impairment status [118,119] and may not have been sensitive enough to identify individuals exhibiting a-MCI/AD pathology, which may help to explain the lack of associations found with a-MCI status in this study. Third, CSHA norms for FAS were used for the Controlled Oral Word Associations Test (CFL), which may have minimally influenced the derived scale scores. Finally, some measures were used for both cognitive status classification and as outcome measures in select analyses (e.g., RAVLT_A7, Block Design). Circularity is a concern when the cognitive status subgroups are examined simultaneously. However, as significant findings were identified not only between but also within subgroups, a circularity bias is not a significant concern.

Overall, the findings of this study reveal several interesting and novel associations involving peripheral inflammation and cognitive status and performance in the context of a-MCI and mild/moderate AD. As aberrant inflammatory processes appear to be present at early stages of cognitive impairment where the underlying disease pathology is likely not very advanced, targeting inflammation at this stage with suitable interventions could be beneficial in terms of delaying and reducing clinical progression. Relatedly, recent research has linked short-term multi-modal physical activity with the reduction of peripheral IL-6 and TNF-α protein levels alongside concomitant improvements in cognitive function (i.e., improvements on the Montreal Cognitive Assessment) in individuals with MCI [109], highlighting the potential utility of environmental interventions (i.e., physical fitness) for targeting molecular mechanisms involved

in AD pathogenesis. Finally, although it is important to understand unique contributions of individual risk factors, integration and interactions with other known biological (i.e., APOE ε4 status) ([69]) and lifestyle (i.e., vascular burden) [70] risk or protective (i.e., APOE ε2 status) factors is an important future step in prediction modeling, consistent with the multi-factorial nature of AD pathogenesis.

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Tables

Table 1. Research questions.

Research Questions	Statistical Analyses	Description of Analyses
RQ#1: Cognitive status group differences in inflammation	Analyses of Covariance Inflammatory marker = Cognitive status + meds + gender + e	Examining cognitive status group differences in mean protein levels
RQ#2a: Inflammation and cognitive performance across cognitive status groups (n = 49)	Regression Analyses Cognitive Performance = Inflammatory marker + meds + gender + e	Examining the associations of inflammatory protein levels on cognitive performance, independent of covariates
RQ#2b: Inflammation and cognitive performance within cognitive status groups (HC, a-MCI, AD separately)	Regression Analyses Cognitive Performance = Inflammatory marker + meds + gender + e	Examining the associations of inflammatory protein levels on cognitive performance, within cognitive status subgroups

Notes. HC, Healthy Controls; a-MCI, Amnestic Mild Cognitive Impairment; AD, Alzheimer's disease; meds, anti-inflammatory medication use; e, error; n, sample size

Table 2. Neuropsychological Tests.

Cognitive Domain	Neuropsychological Test
Global Cognition	3MS
Attention	WAIS-R Digit Span Total
Working Memory	WAIS-R Digit Span Backwards
Memory	Benton Visual Retention Task (Multiple Choice version) Rey Auditory Verbal Learning Task (Total score and A7)
Executive Function	WAIS-R Similarities Trails B
Visual-construction	WAIS-R Block Design
Language	Controlled Oral Word Associations Test Animal Naming
Processing Speed	WAIS-R Digit Symbol Trails A

Notes. A7, RAVLT long delayed trial; WAIS-R, Wechsler Adult Intelligence Scale Revised; 3MS, Modified Mini Mental Examination

Table 3. Descriptive information for sample.

	HC	a-MCI	p^1	AD	p^2
N					
	23	20		6	
Age					
	73.17 (5.83)	73.80 (5.26)	.35	77.43 (5.86)	.05
Gender (W)					
	73.9%	50%	.06	33.3%	.04
Education					
	14.89 (2.76)	15.15 (4.11)	.40	13.29 (5.86)	.04
IL-6	11.19 (16.06)	14.44 (22.34)		23.63 (52.34)	
TNF-α	35.80 (55.44)	63.45 (123.32)		114.81 (255.25)	
MIF	433.97 (227.08)	509.33 (481.87)		740.64 (680.58)	
MIG	279.85 (248.14)	383.80 (445.79)		681.96 (1207.92)	
3MS	97.48 (3.25)	91.60 (4.57)		68.50 (10.50)	
Digit Span-T	15.52 (3.64)	14.80 (3.12)		10.00 (2.00)	
Digit Span-B	6.91 (2.00)	6.80 (1.94)		4.33 (1.63)	
BVRT-MC	13.96 (.83)	12.40 (1.85)		8.83 (4.07)	
RAVLT-T	44.39 (7.13)	31.05 (5.83)		15.50 (7.56)	
RAVLT-A7	9.48 (2.57)	3.60 (2.82)		1.17 (1.17)	
Similarities	11.78 (1.31)	10.90 (1.55)		8.33 (5.99)	
Trails B	81.06 (36.83)	109.46 (42.47)		321.00 (64.75)	
Block Design	14.83 (4.55)	15.10 (5.39)		9.17 (8.28)	
COWAT	43.95 (10.36)	37.65 (13.71)		25.67 (10.58)	
Animal Naming	20.30 (4.75)	18.05 (4.24)		8.33 (3.67)	
Digit Symbol	47.96 (12.25)	39.80 (11.05)		21.17 (9.24)	

Notes. N, Sample size; HC, Healthy Controls; a-MCI, Amnestic Mild Cognitive Impairment; p^1, comparison between H and a-MCI groups (1-tailed); p^2, comparison between H and AD groups (1-tailed); Age, education, IL-6, TNF-α, MIF and MIG data presented as Average (Standard Deviation); RAVLT-T, Rey Auditory Verbal Learning Task total score; RAVLT A7, RAVLT long delayed trial; 3MS, Modified Mini Mental Examination; Digit Span-T, WAIS-R Digit Span Total; Digit Span-B; WAIS-R Digit Span Backwards; BVRT-MC, Benton Visual Retention Task multiple choice version; COWAT, Controlled Oral Word Association Test (CFL);samples (n=49) included are samples with readings for all 4 pro-inflammatory proteins in pg/mL

Table 4. Analyses of covariance: cognitive status group differences in pro-inflammatory protein levels

Outcome	Covariates	Predictor	Mean Diff (A-B)	SE	95% CI	p
IL-6	--	HC vs MCI	-3.26	7.66	-18.67 – 12.16	.34
	--	HC vs AD	-12.44	11.48	-35.55 – 10.67	.14
	Gender	HC vs MCI	-3.26	7.60	-18.58 – 12.07	.34
	Gender	HC vs AD	-12.44	11.39	-35.41 – 10.5	.14
	Inflam. Meds	HC vs MCI	-3.26	7.42	-18.23 – 11.72	.33
	Inflam. Meds	HC vs AD	-12.44	11.13	-34.89 – 10.01	.14
TNF-α	--	HC vs MCI	-27.65	37.24	-102.60 – 47.31	.23
	--	*HC vs AD*	*-79.01*	*55.83*	*-191.39 – 33.38*	*.09*
	Gender	HC vs MCI	-27.65	36.78	-101.82 – 46.53	.23
	Gender	*HC vs AD*	*-79.01*	*55.15*	*-190.22 – 32.21*	*.08*
	Inflam. Meds	HC vs MCI	-27.65	36.03	-100.31 – 45.02	.22
	Inflam. Meds	*HC vs AD*	*-79.01*	*54.03*	*-187.96 – 29.95*	*.08*
MIF	--	HC vs MCI	-75.36	126.40	-329.79 – 179.08	.28
	--	*HC vs AD*	*-306.66*	*189.52*	*-688.14 – 74.82*	*.06*
	Gender	HC vs MCI	-75.36	123.42	-324.25 – 173.54	.28
	Gender	HC vs AD	-306.66	185.04	-679.84 – 66.51	.05*
	Inflam. Meds	HC vs MCI	-75.36	130.11	-337.74 – 187.02	.29
	Inflam. Meds	*HC vs AD*	*-306.66*	*195.08*	*-700.07 – 86.74*	*.06*
MIG	--	HC vs MCI	-103.95	158.91	-423.81 – 215.91	.26
	--	HC vs AD	-402.11	238.25	-881.70 – 77.48	.05*
	Gender	HC vs MCI	-103.95	154.86	-416.25 – 208.35	.25
	Gender	HC vs AD	-402.11	232.19	-870.36 – 66.14	.05*
	Inflam. Meds	HC vs MCI	-103.95	154.06	-414.64 – 206.74	.25
	Inflam. Meds	HC vs AD	-402.11	230.99	-867.95 – 63.73	.05*

Notes. p values are all presented as one-tailed; HC, Healthy Controls; MCI, Amnestic Mild Cognitive Impairment; AD, Alzheimer's disease; Outcome variable raw scores (continuous) were used in the regression models; the sample size used was the stable sample size (n=49) where individuals included had data for all 4 inflammatory proteins in pg/mL; Italicized findings represent non-significant trends; * denotes significant findings; Anti-Inflam. Meds, self-reported current use of anti-inflammatory medications (more than just 81mg of aspirin 1x/day); A-B, HC minus MCI or AD, where lower values indicate higher inflammation in the MCI or AD group, where applicable.

Table 5. Cognitive performance as a function of individual differences in pro-inflammatory protein levels

Predictor	Covariates	Outcome	β	SE	95% CI	p
IL-6	--	*3MS*	*-.09*	*.06*	*-.21 - .03*	*.08*
	--	RAVLT_A7	-.03	.02	-.08 - .02	.13
	Gender	*3MS*	*-.09*	*.06*	*-.21 - .03*	*.07*
	Gender	RAVLT_A7	-.03	.02	-.08 - .02	.12
	Anti-Inflam. Meds	*3MS*	*-.08*	*.06*	*-.21 - .04*	*.08*
	Anti-Inflam. Meds	RAVLT_A7	-.03	.02	-.08 - .02	.14
TNF-α	--	3MS	-.02	.01	-.05 - <.01	.03*
	--	RAVLT_A7	-.01	.01	-.02 - <.01	.05*
	Gender	3MS	-.02	.01	-.05 - <.01	.03*
	Gender	RAVLT_A7	-.01	.01	-.02 - <.01	.05*
	Anti-Inflam. Meds	3MS	-.02	.01	-.05 - <.01	.03*
	Anti-Inflam. Meds	*RAVLT_A7*	*-.01*	*.01*	*-.01 - <.01*	*.06*
MIF	--	3MS	<-.01	<.01	-.01 - <.01	.14
	--	RAVLT_A7	<-.01	<.01	-<.01 - <.01	.24
	Gender	3MS	<-.01	<.01	-.01 - <.01	.19
	Gender	RAVLT_A7	<-.01	<.01	-<.01 - <.01	.34
	Anti-Inflam. Meds	3MS	<-.01	<.01	-.01 - <.01	.14
	Anti-Inflam. Meds	RAVLT_A7	<-.01	<.01	-<.01 - <.01	.24
MIG	--	3MS	-.01	<.01	-.01 - <.01	.03*
	--	*RAVLT_A7*	*<-.01*	*<.01*	*-<.01 - <.01*	*.08*
	Gender	3MS	-<.01	<.01	-.01 - <.01	.04*
	Gender	*RAVLT_A7*	*-<.01*	*<.01*	*-<.01 - <.01*	*.08*
	Anti-Inflam. Meds	3MS	-<.01	<.01	-.01 - <.01	.04*
	Anti-Inflam. Meds	*RAVLT_A7*	*-<.01*	*<.01*	*-<.01 - <.01*	*.08*

Notes. p values are all presented as one-tailed; β, unstandardized beta co-efficient representing change in cognitive performance for every one unit increase in protein level (pg/mL); SE, standard error; 95% CI, 95% confidence interval; Outcome variable raw scores (continuous) were used in the regression models; the sample size used was the stable sample size (n=49) where individuals included had data for all 4 inflammatory proteins; Italicized findings represent non-significant trends; * denotes significant findings; Anti-Inflam. Meds, self-reported current use of anti-inflammatory medications.

Table 6. Predicting cognitive performance as a function of individual differences in pro-inflammatory protein levels for the a-MCI group.

Predictor	Covariates	Outcome	β	SE	95% CI	p
IL-6	--	3MS	-.09	.04	-.18 - .01	.03*
	--	RAVLT_A7	-2.40	1.16	-4.85 - .05	.03*
	--	Block Design	-.12	.05	-.22 - -.01	.02*
	Gender	3MS	-.09	.05	-.19 - .01	.04*
	Gender	RAVLT_A7	-2.67	1.18	-5.16 - -.18	.02*
	Gender	Block Design	-.08	.04	-.17 - .01	.04*
	Anti-Inflam. Meds	3MS	-.09	.05	-.20 - .02	.05*
	Anti-Inflam. Meds	RAVLT_A7	-2.35	1.20	-4.89 - .18	.03*
	Anti-Inflam. Meds	Block Design	*-.08*	*.05*	*-.19 - .04*	*.09*
TNF-α	--	3MS	-.02	.01	-.04 – .01	.01*
	--	RAVLT_A7	-2.80	1.14	-5.14 - -.46	.01*
	--	Block Design	-.02	.01	-.04 - .01	.03*
	Gender	3MS	-.02	.01	-.04 - -.01	.01*
	Gender	RAVLT_A7	-2.75	1.17	-5.21 - -.29	.02*
	Gender	Block Design	*-.01*	*.01*	*-.03 - .01*	*.08*
	Anti-Inflam. Meds	3MS	-.02	.01	-.04 - -.03	.01*
	Anti-Inflam. Meds	RAVLT_A7	-2.91	1.22	-5.48 - -.34	.02*
	Anti-Inflam. Meds	Block Design	*-.08*	*.05*	*-.19 - .04*	*.08*
MIG	--	3MS	-.01	.01	-.01 - .00	.02*
	--	RAVLT_A7	-3.00	1.03	-5.15 - -.83	.01*
	--	Block Design	-.01	.01	-.01 - .00	.03*
	Gender	3MS	-.01	.01	-.01 - .00	.03*
	Gender	RAVLT_A7	-2.96	1.05	-5.15 - -.76	.01*
	Gender	Block Design	*-.01*	*.01*	*-.01 - .01*	*.07*
	Anti-Inflam. Meds	3MS	-.01	.01	-.01 - .01	.04*
	Anti-Inflam. Meds	RAVLT_A7	-3.13	1.13	-5.52 - -.75	.01*
	Anti-Inflam. Meds	Block Design	-.01	.01	-.01 - .01	.13
MIF	--	3MS	.01	.01	-.01 - .01	.38
	--	RAVLT_A7	.26	1.24	-2.34 – 2.84	.42
	--	Block Design	-.01	.03	-.01 - .01	.36
	Gender	3MS	.01	.02	-.01 - .01	.32
	Gender	RAVLT_A7	.00	.01	-.01 - .01	.45
	Gender	Block Design	.00	.01	-.01 - .01	.43
	Anti-Inflam. Meds	3MS	.01	.02	-.01 - .01	.31
	Anti-Inflam. Meds	RAVLT_A7	.00	.01	-.01 - .01	.48
	Anti-Inflam. Meds	Block Design	.00	.01	-.01 - .01	.47

Notes. p values are all presented as one-tailed; β, unstandardized beta co-efficient; SE, standard error; 95% CI, 95% confidence interval; Outcome variable raw scores (continuous) were used in the regression models; Predictor variables for 3MS and BD models were continuous in nature; Predictor variables for RAVLT_A7 models were dichotomized at their 50th percentile in terms of their frequency in the MCI group for each protein separately (i.e., the cut-point for high vs low levels were 6.84, 20.77, 195.78 and 273.26 pg/mL for IL-6, TNF-α, MIG and MIF respectively); 3MS, Modified Mini-Mental Examination; RAVLT_A7, RAVLT Delayed Memory; Block Design, WAIS-R, Block Design task; Anti-Inflam. Meds, current consumption of anti-inflammatory medication (more than 81mg of aspirin daily); the sample size used was the stable sample size (n=20) where individuals included had data for all 4 inflammatory proteins; * denotes significant findings; italicized findings denote non-significant trends

Chapter 3: Summary

Identification of biological risk factors that predict age-related cognitive decline, impairment and dementia was the programmatic focus of my doctoral research. I have contributed to studies that have further developed and strengthened the BioAge concept by identifying within-person dynamic coupling between biological/physiological processes and cognitive performance [5]. Further, I have critically reviewed current operationalization's of developmental time and proposed new multi-determined, mechanism-based (e.g., vascular health and inflammation) directions in cognitive aging research [39]. Building upon this theoretical enterprise, I contributed to multiple studies that championed for a focus on mechanism-based markers and lead to the identification of novel self-reported and objectively measured vascular risk factors ([70], DeCarlo et al., 2010, 2016, unpublished findings) and genetic polymorphisms [69,70] linked to greater risk of impaired cognitive status, stability (i.e., HC conversion to MCI) and poorer cognitive performance in late-life. The current dissertation project is predicated on the substantial literature highlighting the important role of neuro-inflammation on brain function, further builds upon the proposed mechanism-based directive for future research on predictive models for cognitive impairment and dementia in late life [39], and utilizes my previous knowledge of the role of innate immunity and inflammation in disease risk [121–124].

This unique investigation examined whether plasma levels of the pro-inflammatory proteins IL-6, TNFα, MIF and MIG were associated with a-MCI or AD, compared to HC, and were associated with neuropsychological performance independent of or within cognitive status groups. Results provide evidence that elevated plasma pro-inflammatory proteins are associated with cognitive status (HC vs AD), are associated with cognitive performance regardless of cognitive status and are associated with worse cognitive performance in a-MCI at cross-section.

These results are novel findings within the cognitive aging research, particularly in light of the innovative methodology utilized and approach taken, including; clinical classification of a-MCI and mild/moderate AD, utilizing a comprehensive neuropsychological battery, employing sensitive methods to detect low expressing systemic inflammatory proteins, targeting biological indicators using relatively non-invasive and cost effective approaches, the examination of cognitive performance within cognitive status groups (i.e., a-MCI), and controlling for covariates that can directly impact inflammatory protein levels.

The present findings not only further substantiate literature linking inflammatory processes with Alzheimer's pathology, but extend this knowledge base by contributing to the understanding of how systemic inflammation is associated with neuropsychological performance, irrespective of, and within cognitive status groups. Higher inflammatory protein levels were associated with poorer performance on several cognitive tasks across individuals and irrespective of their cognitive status. Most interestingly, among those with a clinical classification of a-MCI, higher peripheral inflammation was associated with poorer verbal memory and global cognitive performance, a finding that, to the best of our knowledge, has not been demonstrated in the literature to date. This association suggests that those already experiencing mild cognitive impairments (and arguably are likely experiencing some level of aberrant neuro-inflammatory cascades) with a higher inflammatory load may be at a greater risk for experiencing poorer cognitive functioning, further linking higher peripheral pro-inflammatory protein levels with compromised cognitive function. This possibility is further substantiated by the lack of associations found between inflammatory protein levels and cognitive performance in HC's. Finally, the link between higher peripheral inflammation and specific cognitive tests in individuals with a-MCI may provide some insight into the brain-region

specificity of aberrant inflammatory processes in the early stages of neurodegeneration, with long-term memory consolidation functions of the temporal lobe being strongly (and expectedly) implicated in this study.

To advance our understanding of the causes of, and contributors to, age-related cognitive deficits and to facilitate prediction, the manner in which development is operationalized must move away from the sole use of CA. This investigation highlighted the importance of incorporating systemic inflammatory markers in predictive models of cognitive change. However, univariate investigations do not reflect the multiple risk and protective factors (i.e., genetic, biological, environmental) thought to interact and influence late-life cognitive function and predispose individuals to pathological cognitive decline over time. Given that a diversity of biomarker predictors have been shown to exert differential influences on cognitive performance, the search for single causal mechanisms of age-related declines in cognition appears less tenable than multi-causal, interactive explanations. As such, many investigations into cognitive decline have utilized multivariate models, such as the construction of a biological aging score [13,15] and prediction of dementia risk [125–127], and death [12]. Further, as most biological processes are dynamic and change at different rates across individuals, appropriate longitudinal analytic approaches are required for the accurate measurement of such processes across time, settings and individuals [128,129]. Cross-sectional findings should be followed-up with longitudinal investigations to assess the utility of inflammatory markers for predicting individuals who progress to mild/moderate stages of AD, as longitudinal analyses may be the most precise way to understand how levels of peripheral cytokines change in the context of progression of cognitive symptoms (c.f. [116]). Longitudinal follow-up of individuals with a-MCI with high peripheral

inflammation would be particularly helpful to better understand the associations between peripheral inflammation in a-MCI, chronicity of cognitive status and conversion to AD.

Despite the focus on aetiological mechanisms, moderators of cognitive health must be considered. Exposure to environmental stressors, epigenetic modifications, disease comorbidities or general health ailments, for example, likely play instrumental roles in late-life cognitive function. For example, exposure to environmental stress has been linked to reactive oxygen species (ROS) accumulation and plaque pathology in AD-like brains [130] and pesticide exposure has been linked to increased dementia risk [131]. Further, research on frailty highlights that a diverse range of health deficits, themselves not known for being aetiological risk factors for dementia, can be combined as a multivariate frailty index to identify those at risk for dementia or death [127]. Overall health may predispose those already at risk for dementia to an accelerated disease course, and as such, may represent an early disease modifying factor. To the extent that BioAge models index moderators (e.g., general health) as well as causal factors (e.g., inflammatory status, vascular health), future BioAge models may more accurately index the true multi-determined, interactive nature of cognitive function and be able to identify individuals at risk for dementia where suitable and specific interventions ([109,112]) could be implemented to lessen the impact of cognitive impairment on patients, their families and society.

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Appendix A

PREVENT Classification Profile Sheet

PREVENT Classification Profile Sheet

		Cannot Assess	None	Quest.	Mild (1)	Mod. (1.5)	Sev (2 S.D.)
A.	**Memory**						
	1) Visual Memory impairment (Benton)	___	___	___	___	___	___
	2) Verbal Memory Impairment (RAVLT Total)	___	___	___	___	___	___
	3) Verbal Memory Impairment (RAVLT A7)	___	___	___	___	___	___
B.	**Other**						
	1) Executive Function						
	a. Abstract Thinking (WAIS-R Sim)	___	___	___	___	___	___
	b. Switching (Trails B)	___	___	___	___	___	___
	2) Aphasia						
	a. Word fluency	___	___	___	___	___	___
	b. Animal fluency	___	___	___	___	___	___
	3) Apraxia						
	a. WAIS-R Block Design	___	___	___	___	___	___
C.	**Functional Impairment**						
	a. Do **memory** impairments cause Significant impairment in social or occupational functioning?						
	1. Subjective, or			Yes _____ No_____			
	2. Measured (ADLs)			Yes _____ No_____			
	b. Do **"other"** impairments cause significant impairment in social or occupational functioning?						
	1. Subjective impairment, or			Yes _____ No_____			
	2. Measured impairment (ADLs)			Yes _____ No_____			
	c. Do **memory** impairments represent sig. declines from previous levels?						
	1. Subjective impairment			Yes _____ No_____			
	d. Do **"other"** impairments represent sig. declines from previous levels?						
	1. Subjective impairment			Yes _____ No_____			
D.	Is the disease course gradual and progressive?			Yes _____ No_____			
E.	The cognitive deficits are not due to other deficits/disorders, such as cerebrovascular disease, Parkinson's Disease, brain tumor, folic acid deficiency			Yes _____ No_____			
F.	The deficits do not occur exclusively during the course of delirium			Yes _____ No_____			
G.	The disturbance is not better accounted for by another Axis 1 disorder, such as MDD or Schizophrenia			Yes _____ No_____			

Collateral Interview

PREVENT Classification: Clinical Interview
DSM-IV-TR Criteria

Background/Qualitative Information

1. Age of participant at time of diagnosis from medical doctor: _____

2. Individual's/proxy's perception of when symptoms first appeared: _____
_____

3. Description of Disease Course from participant/proxy (gradual and continual vs fast):
_____
_____

4. Participants biological family history of cognitive impairment (if any): _____
_____

5. Does the participant have a complaint about their memory? Yes ___ No ___

Description:_____

6. Does informant/proxy have a complaint of participant's memory difficulties? Yes ___ No___

Description: _____

7. Does the participant/informant believe **memory impairments** cause significant impairment in social or occupational functioning?
Yes ___ No ___ Who _____

Description: _____

8. Does the participant/informant believe **"other" impairments** (executive, language, constructional) cause significant impairment in social or occupational functioning? Yes ___ No ___ Who_____
Description: _____

9. Does the participant/informant believe **memory impairments** represent significant declines from previous levels?
Yes ___ No ___ Who _____
Description: _____

10. Does the participant/informant believe **"other" impairments** (executive, language, constructional) represent significant declines from previous levels?
Yes ___ No ___ Who _____
Description: _____

11. Is general cognitive functioning essentially preserved (clinician's judgement)? Yes ___ No ___

12. Are functional activities largely intact? (i.e., no impairments in social or occupational functioning **AND** no declines from previous levels) Yes ___ No ___