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CSF markers of Alzheimer's pathology and microglial activation are associated with altered white matter microstructure in asymptomatic adults at risk for Alzheimer's disease

Kelsey E Melah^a, Sharon Yuan-Fu Lu^b, Siobhan M Hoscheidt^a, Andrew L Alexander^b, Nagesh Adluru^b, Daniel J Destiche^b, Cynthia M Carlsson^a, Henrik Zetterberg^{c,d}, Kaj Blennow^{c,e}, Ozioma C Okonkwo^{a,f}, Carey E Gleason^{a,g}, N Maritza Dowling^{a,h}, Lisa C Bratzkeⁱ, Howard A Rowley^j, Mark A Sager^f, Sanjay Asthana^{a,g}, Sterling C Johnson^{a,f,g}, and Barbara B Bendlin^{a,f}

^aWisconsin Alzheimer's Disease Research Center, University of Wisconsin School of Medicine and Public Health, 600 Highland Avenue J5/1 Mezzanine, Madison, WI 53792

^bWaisman Laboratory for Brain Imaging and Behavior, Madison, WI, USA.

^cInstitute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, SE-431 80 Mölndal, Sweden

^dUCL Institute of Neurology, Queen Square, London WC1N 3BG, UK

^eThe Torsten Söderberg Professorship at the Royal Swedish Academy of Sciences

^fWisconsin Alzheimer's Institute, School of Medicine and Public Health, 7818 Big Sky Dr., Madison, WI 53719

^gGeriatric Research Education and Clinical Center, Wm. S. Middleton Memorial Veterans Hospital, 2500 Overlook Terrace, Madison, WI 53705

^hDepartment of Biostatistics and Medical Informatics, University of Wisconsin School of Medicine and Public Health, 600 Highland Avenue, Madison, WI 53705

ⁱSchool of Nursing, Wisconsin School of Medicine and Public Health, Madison, WI

^jDepartment of Neuroradiology, School of Medicine and Public Health, 600 Highland Avenue, Madison, WI 53705

Abstract

Background—The immune response in Alzheimer's disease (AD) involves activation of microglia which may remove β -amyloid. However, overproduction of inflammatory compounds may exacerbate neural damage in Alzheimer's disease. AD pathology accumulates years before diagnosis, yet the extent to which neuroinflammation is involved in the earliest disease stages is unknown.

Objective—To determine whether neuroinflammation exacerbates neural damage in preclinical AD.

Methods—We utilized cerebrospinal fluid (CSF) and magnetic resonance imaging collected in 192 asymptomatic late-middle-aged adults (mean age=60.98 years). Neuroinflammatory markers chitinase-3-like protein 1 (YKL-40) and monocyte chemoattractant protein-1 (MCP-1) in CSF were utilized as markers of neuroinflammation. Neural cell damage was assessed using CSF neurofilament light chain protein (NFL), CSF total tau (T-Tau), and neural microstructure assessed with diffusion tensor imaging (DTI). With regard to AD pathology, CSF A β ₄₂ and tau phosphorylated at threonine 181 (P-Tau₁₈₁) were used as markers of amyloid and tau pathology, respectively. We hypothesized that higher YKL-40 and MCP-1 in the presence of AD pathology would be associated with higher NFL, T-Tau, and altered microstructure on DTI.

Results—Neuroinflammation was associated with markers of neural damage. Higher CSF YKL-40 was associated with both higher CSF NFL and T-Tau. Inflammation interacted with AD pathology, such that greater MCP-1 and lower A β ₄₂ was associated with altered microstructure in bilateral frontal and right temporal lobe and that greater MCP-1 and greater P-Tau₁₈₁ was associated with altered microstructure in precuneus.

Conclusion—Inflammation may play a role in neural damage in preclinical AD.

Keywords

Alzheimer's disease; Inflammation; Diffusion Tensor Imaging; Cerebrospinal Fluid Proteins

1. INTRODUCTION

Inflammation is a well-established feature of Alzheimer's disease (AD) [1,2]. Several pro-inflammatory cytokines, chemokines, and complement proteins are elevated in the AD brain and both human and animal studies suggest inflammation may occur early in the AD process [3–7]. The underlying cause and temporal sequence of inflammation in relation to AD pathology is not entirely known, although it may occur in response to accumulation of amyloid pathology [8–10]. The inflammatory response in AD is largely mediated by microglia, which are activated to their proinflammatory state via a cytokine-signaling cascade [11]. While the immune response in AD may be necessary to clear amyloid, inflammatory mediators released by chronically active microglia may also contribute to neuronal damage by causing injury and toxicity to neurons [8,12]. In cell culture, microglia activated by β -amyloid have been shown to release reactive oxygen species, TNF- α , and several other molecules that are known to induce apoptosis [13,14]. While this phenomena has been well studied in vitro, the extent to which neuroinflammation may affect neural tissue in vivo is presently unclear, particularly in early disease stages. Understanding the effect of inflammation in preclinical AD could inform the timing of anti-inflammatory therapies, which could play a role in disease prevention.

The primary aim of the current study was to determine the relationship between microglial mediated neuroinflammation and neural damage in asymptomatic adults enriched for AD risk factors including parental family history of AD and apolipoprotein E ϵ 4 (*APOE* ϵ 4) genotype. Two cerebrospinal fluid (CSF) markers were utilized as proxies of

neuroinflammation, chitinase-3-like protein 1 (YKL-40) and monocyte chemoattractant protein-1 (MCP-1). These two proteins are elevated in pre-clinical AD, mild cognitive impairment (MCI), and early AD [4,15–18]. MCP-1 is a potent chemokine, which in the brain serves primarily to attract microglial and peripheral immune cells to sites of inflammation and may also stimulate microglia to change from resting to activated morphology [19]. In turn, YKL-40 is produced by activated microglia, and has been previously associated with reduced cortical thickness and increased CSF total tau (T-Tau) protein in preclinical AD [20].

In order to assess neural damage, outcome markers were derived from CSF and magnetic resonance imaging (MRI). CSF markers included neurofilament light chain protein (NFL) and T-Tau. Both NFL and T-Tau are markers of structural proteins of neurons predominantly localized in large caliber myelinated and thin unmyelinated axons respectively. Because these proteins are released into the CSF from damaged or dying neurons, NFL and T-Tau have been used extensively as neural injury markers in a variety of neurodegenerative diseases [21–24]. MRI based outcomes included hippocampal volume derived from T1-weighted imaging, and whole-brain maps of tissue microstructure derived from diffusion tensor imaging (DTI). Medial temporal lobes are affected early in AD, and hippocampal volume is reduced in AD, MCI, and prodromal stages of the disease [25,26]. In turn, DTI is sensitive to diffusion of water molecules and has been used to assess neural microstructure in upwards of 10,000 human and animal studies. Of relevance to AD, DTI studies have shown neural damage across the spectrum of AD, MCI, and preclinical disease [27–38]. The two summary DTI measures used in this study were fractional anisotropy (FA), a measure of directional water diffusion that is highly sensitive to microstructural features including axonal density, diameter, and myelination, and mean diffusivity (MD), a measure of isotropic diffusion that is sensitive to cellular structure, necrosis, and edema [39,40]. In order to assess the relationship between neuroinflammation and neural microstructure across the brain, we performed a whole brain voxel wise analysis of the DTI maps. We hypothesized that greater neuroinflammation would be associated with higher CSF NFL and T-Tau, in addition to lower hippocampal volume, as well as lower FA and higher MD as shown on DTI.

Given that neuroinflammation may exacerbate neural injury in the presence of existing AD pathology, we also tested for interactions between neuroinflammation and AD pathology—specifically interactions with amyloid and neurofibrillary tangle pathology as measured in CSF. CSF A β ₄₂ and P-Tau₁₈₁ are markers of amyloid plaque and neurofibrillary tangle pathology respectively, with lower A β ₄₂ indicating greater amyloid deposition and higher P-Tau₁₈₁ indicating greater tangle pathology. High P-Tau₁₈₁ and low A β ₄₂ in CSF have been associated with atrophy, cortical thinning, and altered white matter microstructure in preclinical AD [41–45], but the extent to which neuroinflammation may moderate these effects is unknown. Given the potential negative effects of inflammation on neural health, we hypothesized that the negative effects of β -amyloid and neurofibrillary tangle pathology on tissue microstructure would be greater among individuals with higher inflammation.

2. MATERIALS AND METHODS

Study procedures were approved by the University of Wisconsin Health Sciences Institutional Review Board and were in accordance with U.S. federal regulations. All participants provided written informed consent.

2.1 Participants

Participants were asymptomatic late-middle-aged adults ($N = 192$, mean age = 60.98 years, $SD = 7.55$, range = 46-85 years) from the Wisconsin Registry for Alzheimer's Prevention (WRAP) study and the Wisconsin Alzheimer's Disease Research Center (ADRC) clinical core who underwent brain imaging and lumbar puncture as part of studies on memory, aging, and preclinical AD. Participants were selected from these larger studies based on available DTI and CSF data, which only a subset of participants have available. Both the WRAP and Wisconsin ADRC cohorts comprise well-characterized and longitudinally followed participants who are either positive or negative for parental history of AD. Positive parental family history of AD classification was defined as having one or both parents with AD as determined by a validated interview [46] or autopsy-confirmed or probable AD as outlined by research criteria [47,48], and reviewed by a multidisciplinary diagnostic consensus panel. Detailed medical history and phone interviews were conducted to confirm family history negative participants. Absence of family history of AD required that the participant's father survive to at least age 70 years and the mother to age 75 years without diagnosis of dementia or cognitive deterioration. Family history was classified as a binary variable. *APOE* $\epsilon 4$ genotype was performed on a non-fasting blood sample collected at baseline, using standard PCR and DNA sequencing techniques. DNA extracted from whole blood was genotyped with use of a homogeneous Fluorescent Resonance Energy Transfer technology coupled to competitive allele specific PCR (LGC Genomics; Beverly, MA). Genotyping also was performed by NCRAD. There was 100% concordance for *APOE* genotype between these analyses. Participants were categorized using a binary variable as an *APOE* $\epsilon 4$ carrier or non-carrier.

General inclusion criteria consisted of: 1) normal cognitive function determined by comprehensive neuropsychological evaluation and consensus review, 2) negative history of psychiatric or neurological disease or untreated depression, and 3) no history of head trauma. While individuals with major medical illness (such as current malignancy and major organ failure) were not included, 12 of the participants had a diagnosis of Type 2 Diabetes. Given that there was no significant difference in levels of neuroinflammation between these participants and participants without diabetes, they remained in the analysis. Participants were also required to have previously undergone MRI and lumbar puncture for CSF assays.

Participants were excluded from the analysis based on one or more of the following: a) missing CSF data due to inadequate CSF collection for assays ($n=1$), b) unsatisfactory DTI data quality after processing, such as major susceptibility artifact ($n=13$), c) an incomplete or failed DTI acquisition or transfer ($n=11$), d) or abnormal radiological report on study MRI ($n=31$). While not all abnormalities noted on the radiology report required clinical follow-up, we took a conservative approach and removed participants with abnormalities that would affect data processing or interpretation of DTI findings in cerebral white matter (for

example, noted presence of small vessel disease or possible history of infection). Of 248 participants identified for possible inclusion in the study, 192 participants met criteria for inclusion. Overall the sample was largely female, Caucasian, well educated, had a parental family history of AD or were *APOE* $\epsilon 4$ carriers (see Table 1).

2.2 CSF collection and analysis

CSF was collected with a Sprotte 25-or 24-gauge spinal needle at the L3/4 or L4/5 using gentle extraction into polypropylene syringes. Samples were collected in the morning after a 12h fast. Approximately 22mL of CSF were inverted to avoid gradient, gently mixed and centrifuged at 2000g for 10 minutes. Supernatants were frozen in 0.5mL aliquots in polypropylene tubes and stored at -80°C . Samples were analyzed for P-Tau₁₈₁, T-Tau and A β ₄₂ using commercially available enzyme-linked immunosorbent assay (ELISA) methods (INNOTEST assays, Fujirebio, Ghent Belgium) as described previously in detail [49]. MCP-1 levels in CSF were measured using the Meso Scale Discovery technique (MSD Human MCP-1; Meso Scale Discovery, Gaithersburg, MD, USA), and YKL-40 was determined using a sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, Minn., USA). Likewise, CSF NFL was measured with a sandwich ELISA method (NF-light ELISA kit, UmanDiagnostics AB, Umeå, Sweden). Board-certified laboratory technicians who were blinded to clinical diagnosis performed all analyses on one occasion. All samples were analyzed according to protocols approved by the Swedish Board of Accreditation and Conformity Assessment (SWEDAC) using one batch of reagents (intra-assay coefficients of variation <10%). Two participants had P-Tau₁₈₁ or T-Tau levels below the detectable threshold of our assays. They were included in the analysis but were assigned the minimum detectable value for the particular marker (90.8ng/mL for T-Tau, 15.6ng/mL for P-Tau₁₈₁).

2.3 Magnetic Resonance Imaging

The average delay between MRI and lumbar puncture was 6.57 days (SD 19.68). Participants underwent scanning on one of two identical General Electric 3.0 Tesla Discovery MR750 MRI systems with 8 channel head coils and parallel imaging (ASSET). DTI was acquired using a diffusion-weighted, spin-echo, single-shot, echo planar imaging (EPI) pulse sequence in 40 encoding directions with $b = 1300 \text{ s/mm}^2$, and eight non-diffusion weighted ($b = 0$) reference images. The cerebrum was covered using contiguous 2.5 mm thick axial slices, FOV = 24 cm, TR = 8000 ms, TE = 67.8 ms, matrix = 96×96 , resulting in isotropic 2.5 mm^3 voxels. High order shimming was performed prior to the DTI acquisition to optimize the homogeneity of the magnetic field across the brain and to minimize EPI distortions. A T1-weighted volume was acquired in the axial plane with a 3D fast spoiled gradient-echo sequence using the following parameters: inversion time (TI) = 450 ms; repetition time (TR) = 8.1 ms; echo time (TE) = 3.2 ms; flip angle = 12° ; acquisition matrix = $256 \times 256 \text{ mm}$, field of view (FOV) = 256 mm; slice thickness = 1.0 mm. 3D T2-weighted fluid attenuated inversion recovery (FLAIR) scans were acquired in the sagittal plane using the following parameters: TI = 1868 ms; TR = 6000 ms; TE = 123 ms; flip angle = 90° ; acquisition matrix = $256 \times 256 \text{ mm}$, FOV = 256 mm; slice thickness = 2.0 mm, no gap, yielding a voxel resolution of $1 \text{ mm} \times 1 \text{ mm} \times 2 \text{ mm}$.

2.4 MRI Processing

DTI was processed using a customized pipeline, as described in detail in Adluru et al. [50]. Briefly, images underwent eddy current correction, field inhomogeneity correction, and skull stripping using tools from the FMRIB Software Library (FSL) (<http://www.fmrib.ox.ac.uk/fsl/>). Tensor fitting was performed using the University College London, Camino Diffusion MRI Toolkit (<http://cmic.cs.ucl.ac.uk/camino/>). Tensor-based registration was implemented utilizing Diffusion Toolkit for DTI analysis (DTI-TK, <http://dti-tk.sourceforge.net/pmwiki/pmwiki.php>), whereby images were registered to a population specific template created with DTI-TK. FA and MD maps were calculated using DTI-TK. Given that anisotropic water diffusion is less interpretable in gray matter compared to white matter, FA analyses were limited to white matter. A white matter mask was constructed by thresholding the FA template to values > 0.15 . MD analyses were conducted in both gray and white matter, using a brain mask constructed by thresholding the population based MD template image to 0.10. Both masks were visually inspected to ensure inclusion of tissue of interest. The MD template was solely used for constructing the brain mask and as an underlay for results (i.e. not used for normalization). In order to control for cerebrovascular disease and ischemic lesion burden, total white matter hyperintensity (WMH) volume for each participant was determined using Lesion Segmentation Tool (version 1.2.2) implemented in SPM8 [51]. The T1-weighted and T2FLAIR images were processed according to the method detailed in Birdsill et al. [52]. WMH was adjusted for variability in head size by dividing total WMH by intracranial volume to yield a WMH ratio (WMHr). Intracranial volume was calculated using the “reverse brain masking” method detailed in Keihaninejad et al. [53].

Hippocampal volume was calculated using FSL’s (FMRIB Software Library) (<http://www.fmrib.ox.ac.uk/fsl/>) FIRST tool [54]. The shape/appearance models used in FIRST are constructed from manually segmented images provided by the Center for Morphometric Analysis (CMA), MGH, Boston. Hippocampal volumes were corrected for intracranial volume and expressed as a ratio of hippocampal volume to total intracranial volume.

2.5 Statistical Analysis

2.5.1 Effect of Neuroinflammation on CSF markers of neural injury—To test the effect of neuroinflammation and AD pathology on axonal damage, several linear regression models were implemented in SPSS (IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp), where YKL-40, MCP-1, P-Tau₁₈₁, and A β ₄₂ were the predictor variables, and CSF T-Tau and NFL were the dependent variables, controlling for age, sex, family history of AD, *APOE* $\epsilon 4$, and WMHr. We also tested for interactions between AD pathology (P-Tau₁₈₁, and A β ₄₂) and neuroinflammation (YKL-40 and MCP-1) on CSF T-Tau and NFL. NFL was log transformed to correct for a slight right skew. All other variables were approximately normally distributed. To account for type 1 error caused by multiple comparisons, a Bonferroni correction was implemented (the α level of .05 was divided by the number of contrasts performed, i.e. 16), yielding a corrected alpha of $\alpha=0.0031$.

2.5.2 Effect of Neuroinflammation on Hippocampal volume—To test the effect of neuroinflammation and AD pathology on hippocampal volume, linear regression models were implemented in SPSS where YKL-40, MCP-1, P-Tau₁₈₁, and A β ₄₂ were the predictor variables and left and right hippocampal volume were the dependent variables, covarying for age, sex, family history, APOE ϵ status, and WMHr. Additionally we tested for interactions of pathology (A β ₄₂ and P-Tau₁₈₁) and neuroinflammation (YKL-40 and MCP-1) on left and right hippocampal volume. To account for type 1 error caused by multiple comparisons, a Bonferroni correction was implemented (the α level of .05 was divided by the number of contrasts performed, i.e 16), yielding a corrected alpha of $\alpha=0.0031$.

2.5.3 Effect of Neuroinflammation on microstructure: Voxel-wise DTI analysis

—To test the effect of neuroinflammation and AD pathology on tissue microstructure, voxel-wise analyses of FA and MD maps were implemented using statistical parametric mapping software (SPM8; <http://www.fil.ion.ucl.ac.uk/spm/>). Multiple regression models tested the main effects of YKL-40 and MCP-1 on FA and MD across the whole brain, in addition to testing for interactions between YKL-40 and MCP-1 and CSF A β ₄₂ and P-Tau₁₈₁. Models used age, sex, family history, APOE ϵ status, and WMHr as covariates. Results were determined using a cluster extent based thresholding approach, with a primary voxel level threshold of $p < 0.005$. This method offers increased sensitivity to spatially extended signals with moderate effect size [55]. To account for spatial non-stationarity of the DTI maps, we used the random field theory based approach documented in Worsley et al. [56]. The results were further corrected using the Bonferroni method (.05/35), yielding a corrected α of 0.0014. Results that survive Bonferroni correction are indicated with asterisks in Table 2.

Relationship between CSF markers of neural injury and regional microstructure: Voxel-wise DTI analysis

—While primary analyses centered on the effects of neuroinflammation and AD pathology, it was of secondary interest to test whether the CSF and imaging outcome markers of neural injury were associated with each other. Voxel-wise regression models were utilized to assess whether NFL and T-Tau were associated with FA and MD. Additionally, interactions of NFL and T-Tau with YKL-40, MCP-1, A β ₄₂, and P-Tau₁₈₁ were tested. Age, sex, family history, APOE ϵ status, and WMHr were included as covariates. NFL was log₁₀ transformed to correct for slight right skew. As in the preceding analysis, significant results were determined using a cluster extent based thresholding approach, with a primary voxel level threshold of $p < 0.005$, further corrected using the Bonferroni method ($\alpha = 0.0014$). Results that survive Bonferroni correction are indicated with asterisks in Table 2.

3. RESULTS

Effect of Neuroinflammation on CSF markers of neural injury

Neuroinflammation was associated with neural injury. The linear regression analysis implemented in SPSS revealed a significant effect of YKL-40 on CSF NFL and T-Tau concentration. As predicted, higher YKL-40 was associated with higher CSF NFL ($\beta=0.254$, $p<0.001$) as well as higher CSF T-Tau ($\beta=0.553$, $p<0.001$)(Figure 1), suggesting

degeneration of both large caliber and thin unmyelinated axons. MCP-1 was not associated with CSF NFL or T-Tau levels. Markers of AD pathology were also associated with neural injury. Linear regression analyses in SPSS revealed that *higher* $A\beta_{42}$ was associated with higher T-Tau ($\beta=0.244$, $p=0.001$) (Figure 1), although analysis with NFL did not survive Bonferroni correction ($\beta=0.127$, $p=0.032$). Additionally, higher P-Tau₁₈₁ was associated with greater concentration of CSF NFL ($\beta=0.256$, $p<0.001$) (Figure 1) and T-Tau ($\beta=.869$, $p<0.001$). Neuroinflammation did not interact with AD pathology to have an effect on neural injury, i.e. CSF NFL or T-Tau.

Effect of Neuroinflammation on Hippocampal volume

No significant effects of MCP-1, YKL-40, $A\beta_{42}$ or P-Tau₁₈₁ on hippocampal volume were found. No significant interactions of neuroinflammation and Alzheimer's pathology on hippocampal volume were found.

Effect of Neuroinflammation on microstructure: Voxel-wise DTI analysis

Cluster level results of the voxel-wise DTI analysis are presented in Table 2, and are organized into sub-tables by category (main effects, interactions with AD pathology, and interactions with neural injury markers), and are further divided into rows based on predictor variables and outcome measures.

There were no main effects of neuroinflammation on tissue microstructure as indexed by FA and MD (Table 2A); however, neuroinflammation interacted with AD pathology to significantly impact tissue microstructure. Interactions were observed between MCP-1 and $A\beta_{42}$ on MD in the bilateral inferior frontal lobe, right inferior temporal lobe, and left fusiform gyrus (Figure 2, Table 2B). The relationship was such that among participants with lower CSF $A\beta_{42}$ (suggesting cerebral amyloid deposition), higher MCP-1 was associated with higher MD, suggestive of tissue damage. The interaction between YKL-40 and $A\beta_{42}$ did not reach significance.

A voxel-wise analysis of FA also revealed an interaction between MCP-1 and CSF P-Tau₁₈₁ (Table 2B). Specifically, among participants with higher CSF P-Tau₁₈₁, higher MCP-1 was associated with higher FA in the right post-central gyrus white matter, right corona radiata, and left anterior internal capsule. In the corresponding MD analysis, this interaction was noted in the left precuneus. In both of these interactions, greater neuroinflammation (high MCP-1) in the presence of greater tau pathology (high CSF P-Tau₁₈₁) was associated with both higher FA and MD. An interaction was also observed between YKL-40 and P-Tau₁₈₁ in the left precuneus (Figure 3, Table 2B), where higher YKL-40 and higher P-Tau₁₈₁ were associated with greater MD. Brain regions where main effects of NFL, T-Tau and P-Tau₁₈₁ were observed differed from the regions where interactions were noted. For example, as is detailed under main effects in Table 2A, higher CSF P-Tau₁₈₁ was associated with higher FA in bilateral thalamus, and higher MD in the right orbitofrontal gyrus and bilateral post-central gyrus.

Relationship between CSF markers of neural injury and regional microstructure: Voxel-wise DTI analysis

Given that our sample was characterized on NFL and T-Tau, proteins that are primarily localized to axons, we conducted voxel-wise analyses to determine whether CSF NFL and T-Tau levels alone, or in combination with neuroinflammation were associated with regional gray and white matter microstructure. The voxel-wise analyses revealed widespread significant associations between CSF NFL and MD across lateral and medial temporal, parietal, and frontal lobes (Figure 4, Table 2A) and between CSF T-Tau and FA in the bilateral thalamus and MD in bilateral precuneus, left cuneus, right fusiform gyrus, and left orbitofrontal gyrus. As detailed in Table 2C, YKL-40 showed a significant interaction with CSF NFL on FA in right internal and external capsule, in addition to effects on MD, with a peak association in right angular gyrus. Here, higher YKL-40 and higher NFL were associated with greater FA and MD. Finally, YKL-40 interacted with CSF T-Tau on FA in the bilateral internal capsule, and on MD in the left cerebellum and left superior frontal gyrus. In both cases, higher NFL or T-Tau along with greater inflammation was associated with higher FA and MD. CSF NFL also interacted with P-Tau₁₈₁, where elevated P-Tau₁₈₁ combined with elevated CSF NFL was associated with higher MD in the right precuneus (Table 2C). Similarly, CSF T-Tau also interacted with P-Tau₁₈₁ in the cuneus, whereby greater neural damage indicated by higher NFL or T-Tau and higher P-Tau₁₈₁ were associated with higher MD. In contrast to the YKL40 effects, MCP-1 did not interact with T-Tau or NFL on either DTI measure.

4. DISCUSSION

Pathological changes associated with AD appear years before the onset of clinically relevant symptoms [57]. Prospective treatments or therapies may be most beneficial in the preclinical stage, before extensive and irreversible neural damage. Microglial activation plays a role in clearance of cellular debris and β -amyloid; however, the associated inflammatory cascade may also contribute to neural damage [12]. In the present study, we assessed for the first time, whether neuroinflammation is associated with preclinical neural damage, particularly in the presence of amyloid and neurofibrillary tangle pathology. As predicted, indicators of a neuroinflammatory response both alone, and in concert with AD pathology, were associated with neural damage. The results of this study suggest that the association between neuroinflammation and neural damage is present in the preclinical stages of AD.

Higher MCP-1 combined with lower A β ₄₂ was associated with altered tissue microstructure in brain regions known to be affected by amyloid pathology, including bilateral frontal cortex, and lateral temporal lobe. MCP-1 is a chemokine expressed in response to inflammatory signals, and primarily serves to attract peripheral immune cells (monocytes) and microglia to sites of inflammation. Cellular studies show that human monocytes and activated microglia produce MCP-1 in response to A β fragments and larger amyloid plaques in order to recruit additional immune cells [58,59]. In humans, MCP-1 is increased in MCI and AD and is predictive of cognitive decline [4,60]. Given that effects of A β ₄₂ on tissue microstructure were only observed among participants with higher MCP-1, the results suggest that elevated inflammation could underlie the tissue alterations. Indeed, microglial

activation along with cell death and degradation of synaptic connections are a distinguishing feature of the post mortem AD brain, compared to cognitively intact controls with comparable levels of plaque and tangle pathology [6].

In addition to relationships with amyloid pathology, we also observed interactions between inflammation and neurofibrillary tangle pathology—specifically a significant interaction between MCP-1 and P-Tau₁₈₁. The combination of high CSF P-Tau₁₈₁ and high CSF MCP-1 was associated with both higher FA in right post-central gyrus white matter and bilateral corona radiata, and higher MD in the left precuneus, suggesting cell loss and potentially selective degeneration of axons in these regions. Lower FA in white matter fiber tracts typically suggests microstructural damage, although axonopathy may also be associated with higher FA due to selective degeneration of axons, particularly in areas of crossing fibers [61]. No studies to date have specifically linked MCP-1 expression to tau reactivity on a cellular level; however, histological studies have found that activated microglia are regionally correlated with extracellular neurofibrillary tangles (NFTs) and dystrophic tangle-bearing neurons even in the early stages of tangle formation [62,63].

In addition to interacting with AD pathology, neuroinflammation alone was also associated with markers of neural damage. Elevated neuroinflammation as shown by higher CSF YKL-40, was associated with higher CSF levels of NFL, a structural protein primarily localized to axons, suggesting axonal damage. YKL-40, a 40kDa glycoprotein, is expressed by activated microglia and is elevated with increasing age [64] and at all stages of AD [15,17,18]. Axonal degeneration has been hypothesized to be an early feature of the AD process [65], although the specific effect of neuroinflammation on axonal degeneration is incompletely characterized [66]. Microglia that have been primed by an inflammatory trigger such as A β , neural injury, or aging may respond by releasing a variety of neurotoxic factors, which further damage surrounding cells [12]. Given that the effects of YKL-40 were observed regardless of A β ₄₂ or T-Tau levels, the association of YKL-40 and neural damage in this study could reflect microglial activation in response to age-associated brain changes [64], or other CNS disorders.

Compared to volumetric imaging, DTI may provide unique insights into early pathological changes. In this study none of the CSF measures were significantly associated with hippocampal volume; however several, especially T-Tau and NFL were associated with the DTI-derived measures of microstructure. Amyloid plaques and neurofibrillary tangle pathology are associated with disruption of cytoskeletal equilibrium, synaptic dysfunction, and axonal degeneration [67–69] while total tau and NFL protein measured in CSF indicate damage to thin unmyelinated and large caliber axons, as damaged cells “leak” tau or NFL protein [70,71]. In this study, higher CSF T-Tau was associated with higher FA and MD in brain regions that are known to undergo pathological changes in early stages of AD including the thalamus, precuneus, cuneus and cingulum. Further, we found a robust relationship between CSF levels of NFL, and MD, involving several brain regions affected in AD, including hippocampus. While higher FA is generally thought of as indicating more intact white matter, several studies suggest that the interpretation of FA may be more complicated. Ryan et al. (2003) reported higher FA among asymptomatic carriers of the PSEN1 mutation that causes autosomal dominant AD, likely due to early and selective loss

of axonal fibers [72]. Our group has also found higher FA in select white matter tracts among individuals with parental family history of AD, in addition to higher FA among individuals with greater amyloid deposition [50,73]. Associations between higher T-Tau and higher FA in this study may suggest selective fiber loss. Another study involving 7T DTI imaging found increased FA in the thalamus, left hippocampus, corpus callosum, anterior commissure, and internal capsule in aged APP/presenilin 1 transgenic mice. These increases in FA were associated with presence of injured neurons, swollen neuronal processes, amyloid plaques, and dystrophic axons [74], features that may explain findings of higher FA in our sample. Overall, these findings suggest axonal loss is an early and measurable feature of preclinical AD.

Amyloid in combination with neuroinflammation was associated with neural injury; however, CSF A β ₄₂ alone did not show a significant association with DTI measures. While amyloid deposition is known to be an early feature of AD, it is thought that the toxic effects on neurons only appear after significant levels of amyloid burden are reached [75]. Another possibility for the lack of direct effect of A β ₄₂ may be due to a dynamic fluctuation in CSF A β ₄₂ that has been observed in preclinical AD. While CSF A β ₄₂ generally declines during the progression of AD, studies in individuals with PSEN1 mutation show elevated CSF A β ₄₂ levels compared to non-carriers in the asymptomatic stage of the disease [76]. The CSF analyses in this study showed that higher CSF A β ₄₂ was associated with higher CSF T-Tau, suggesting greater axonal injury in individuals with higher CSF A β ₄₂. Interestingly, in a recent study by Maia *et al.* [77] researchers noted that in several APP transgenic mice models CSF A β ₄₂ and A β ₄₀ levels showed a slight rise just before the onset of A β deposition. CSF A β ₄₂ levels later dropped in the mice as plaques formed. The association of higher A β ₄₂ with higher T-Tau in our study may reflect this early stage of plaque formation and neural injury. Main effects of CSF A β ₄₂ on DTI may have been below detection threshold in the current study. This notion is supported by findings from our group showing that preclinical amyloid deposition as measured with [C11]PiB-PET was associated with altered microstructure on DTI [73].

There are a few limitations that should be noted. Generalizability of the results may be limited, as the sample was largely Caucasian, female, and highly educated with access to quality healthcare. It is also important to note that the study is cross-sectional. While the results of the current study suggest that neuroinflammatory processes are associated with neural damage, it is important to bear in mind that in a cross sectional study such as this, no determination of causality can be made. While we speculate that neural damage may be incurred by the inflammatory process, it is possible that levels of activated microglia reflect a response to tissue injury itself. Given that amyloid levels alone were not predictive of altered microstructure, this seems a less likely scenario, but longitudinal and animal studies are still needed to further investigate this relationship. Furthermore, while several studies support our postulation that neuroinflammation occurs in response to amyloid and tau pathology, it is important to consider that other studies have suggested that it may also drive pathology by causing accumulation of β -amyloid and tau [8,78]. More research is needed to understand the temporal relationship between inflammation and the development of AD pathology. It should also be noted that the specific biochemical and cellular roles of MCP-1

and YKL-40 are still being investigated [79] and more research is needed to elucidate the specific actions of MCP-1 and YKL-40 in the healthy CNS and in preclinical AD.

Summary

In conclusion, this study suggests that neuroinflammation is associated with neural damage—including elevated levels of structural proteins in CSF and altered regional microstructure as shown on brain imaging—among asymptomatic individuals. The results of this study provide evidence that examining markers of AD pathology together with markers of neuroinflammation may provide greater insight into the pathological processes occurring in preclinical AD. Additional studies are needed to further understand the timing of neuroinflammation in the AD process, and to determine at what disease stage neuroinflammation may be beneficial or deleterious.

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Figure 1. Relationship between neuroinflammation, Alzheimer's pathology, and CSF markers of neural injury

Higher YKL-40 and higher P-Tau₁₈₁ were associated with higher CSF NFL ($\beta=0.254$, $p<0.001^{**}$, and $\beta=0.256$, $p<0.001^{**}$, respectively). Higher YKL-40 and A β_{42} were associated with higher CSF T-Tau ($\beta=0.553$, $p<0.001^{**}$ and $\beta=0.244$, $p=0.001^{**}$, respectively). NFL was log₁₀ transformed to correct for slight right skew. In the scatterplots, transformed NFL and T-Tau were adjusted for all covariates (age, sex, WMHr, *APOE* genotype status, and family history status) and plotted against predictor variables.

Figure 2. MCP-1 $\times$ A β ₄₂ interaction on MD

Higher CSF MCP-1 and lower A β ₄₂ were associated with increased mean diffusivity in several brain regions including the inferior frontal lobe, right fusiform gyrus, and inferior temporal gyri. Results were corrected for non-stationarity using a random field theory based approach with a voxel-level threshold of $p < .005$ and a cluster level threshold of $p < .05$. Variations in the color map correspond to the size of the t-statistic. MD results are overlaid on a T1-weighted image for anatomical detail. Right=Left.

Figure 3. MCP-2 $\times$ P-Tau₁₈₁ interaction on FA (cool) and MD (warm)

Higher CSF MCP-1 and Higher P-Tau₁₈₁ were associated with higher FA in right post central gyrus white matter, right anterior corona radiata, left internal capsule and with higher MD in the left precuneus. Results were corrected for non-stationarity using a random field theory based approach with a voxel level threshold of $p < 0.005$ and a cluster level threshold of $p < 0.05$. Blue clusters indicate FA results, while orange clusters indicate MD results. Variations in color map correspond to the size of the t-statistic. Results are overlaid on a T1-weighted image for anatomical detail. Right=Left.

Figure 4. Relationship between CSF NFL and MD

Higher CSF NFL was associated with greater MD in a variety of brain regions related to AD, including bilateral precuneus, right superior temporal gyrus, right hippocampus, and right superior parietal lobule. Results were corrected for non-stationarity using a random field theory based approach with a voxel level threshold of $p < .005$ and a cluster level threshold of $p < .05$. Variations in color map correspond to the size of the t-statistic. MD results are overlaid on a T1-weighted image for anatomical detail. Right=Left.

Table 1**Participant Demographics**

Age in years[mean, (SD)]	60.98 (7.55)
Sex [n (%)]	
Male	55 (28.9)
Female	137 (71.3)
Ethnicity [n (%)]	
Caucasian	187 (97.4)
African American/Black	2 (1.0)
Hispanic/Latino	0
Other/unknown	3 (1.5)
<i>APOE</i> ε4 status [n (%)]	
Carriers	78 (41)
Non-Carriers	114 (59)
Family history [n (%)]	
Positive	145 (75.5)
Negative	47 (24.5)
Unknown	0
Education in years [mean, (SD)]	16.26 (2.51)
RAVLT total	50.43 (8.65)
RAVLT delay	10.05 (3.07)
MMSE	29.34 (0.886)
Category Fluency (animals)	22.81 (5.39)
Trail Making Test A (seconds)	25.23 (8.56)
Trail Making Test B (seconds)	59.79 (22.23)
YKL-40 (ng/mL)	142760.8 (52093.7)
MCP-1 (ng/mL)	542.8 (135.5)
Aβ ₄₂ (ng/mL)	737.7 (212.4)
T-Tau (ng/mL)	307.3 (126.9)
P-Tau ₁₈₁ (ng/mL)	42.2 (15.1)
NFL (ng/mL)	637.2 (316.4)
WMHr	0.014(.0015)

APOE ε4: Apolipoprotein E ε4; SD: standard deviation; RAVLT: Rey Auditory Verbal Learning Test; MMSE: Mini Mental Status Exam; MCP-1: monocyte chemoattractant protein; Aβ₄₂: 42 residue amyloid beta peptide; T-Tau: Total tau; P-Tau: phosphorylated tau; NFL: neurofilament light chain protein; WMHr: white matter hyperintensity ratio.

Table 2

DTI results

A: Main effects	Anatomical location	k	Peak T	Cluster level P value
P-Tau₁₈₁				
Fractional Anisotropy	thalamus (L,R)	567	5.07	0.015
Mean diffusivity	inferior frontal gyrus (L)	211	4.00	0.031
NFL				
Mean Diffusivity	cuneus (L)	689 5	5.51	<0.001*
	superior parietal lobule (R)	608 5	4.80	<0.001*
	middle frontal gyrus (R)	252 1	4.17	<0.001*
	pre-central gyrus (L)	989	3.96	0.025
	hippocampus (R)	987	4.76	0.029
T-Tau				
Fractional Anisotropy	thalamus (L,R)	433	4.89	0.021
Mean Diffusivity	precuneus (L,R)	144 94	5.54	<0.001*
	cuneus (L)	798	4.34	0.008
	fusiform (R)	437	4.31	0.018
	Inferior orbitofrontal (L)	222	4.43	0.027

B: Inflammation × Alzheimer's marker interactions	Anatomical location	k	Peak T	Cluster level P value
Aβ42x MCP1				
Mean Diffusivity	inferior frontal gyrus (R)	772	4.52	0.002
	inferior temporal gyrus (R)	329	3.46	0.027
	fusiform (L)	357	4.20	0.029
	Inferior frontal gyrus (L)	139	3.71	0.041
P-Tau₁₈₁ × YKL-40				
Mean Diffusivity	precuneus (L)	617	3.46	0.022
P-Tau₁₈₁ × MCP-1				
Fractional Anisotropy	post central gyrus wm (R)	671	4.94	0.0018
	anterior corona radiata (R)	395	4.37	0.013
	anterior internal capsule (L)	557	3.61	0.037
Mean Diffusivity	precuneus (L)	520	3.92	0.034

C: Interactions with neural injury	Anatomical location	k	Peak T	Cluster level P value
P-Tau₁₈₁ × NFL				

C: Interactions with neural injury	Anatomical location	k	Peak T	Cluster level Pvalue
Mean Diffusivity	precuneus (R)	837	3.93	0.005
YKL-40 × NFL				
Fractional Anisotropy	internal/external capsule (R)	777	3.59	0.028
Mean Diffusivity	angular gyrus (R)	1353	6.77	<0.001 *
YKL-40 × T-Tau				
Fractional Anisotropy	internal/external capsule *, cerebral peduncle (L)	1035	4.16	0.008
	cerebral peduncle *, internal/external capsule (R)	688	3.79	0.025
Mean Diffusivity	cerebellum (L)	2567	4.06	0.009
	superior frontal gyrus (L)	1133	3.60	0.022
P-Tau₁₈₁ × T-Tau				
Mean Diffusivity	cuneus (L,R)	757	4.23	0.012

R: right; L: left; MCP-1: monocyte chemoattractant protein; Aβ₄₂: 42 residue amyloid beta peptide; T-Tau: Total tau; P-Tau: phosphorylated tau; NFL: neurofilament light chain protein; WM: white matter;

* cluster peak location,

* survives Bonferroni correction ($\alpha=0.05/36$ contrasts, corrected $\alpha=0.0014$)