Plasma biomarkers of astrocytic and neuronal dysfunction in early- and late-onset Alzheimer's disease

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Abstract

Introduction: We investigated plasma proteomic markers of astrocytopathy, brain degeneration, plasticity, and inflammation in sporadic early-onset versus late-onset Alzheimer's disease (EOAD and LOAD).

Methods: Plasma was analyzed using ultra-sensitive immuno-based assays from 33 EOAD, 30 LOAD, and 36 functionally normal older adults.

Results: Principle component analyses identified 3 factors: trophic (BDNF, VEGF, TGFβ), degenerative (GFAP, NfL), and inflammatory (TNFα, IL-6, IP-10, IL-10). Trophic factor was elevated in both AD groups and associated with cognition and gray matter volumes. Degenerative factor was elevated in EOAD, with higher levels associated with worse functioning in this group. Biomarkers of inflammation were not significantly different between groups and were only associated with age.

Disucssion: Plasma proteomic biomarkers provide novel means of investigating molecular processes *in vivo* and their contributions to clinical outcomes. We present initial investigations of several of these fluid biomarkers, capturing aspects of astrocytopathy, neuronal injury, cellular plasticity, and inflammation in EOAD versus LOAD.

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CONFLICT OF INTERESTS

The authors have declared that no conflict of interest exists.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Keywords

Early-onset Alzheimer's disease; Late-onset Alzheimer's disease; Cerebral small vessel disease; Astrocytopathy; Immune activation; Inflammation; Brain homeostasis; Growth hormones; Exosomes; White matter disease; Neurodegeneration

1 | INTRODUCTION

In the aftermath of negative clinical trials targeting amyloid β (Aβ)[1] and an increasing appreciation of the highly heterogeneous pathophysiology of Alzheimer's disease (AD), there has been a shift toward understanding and targeting alternative pathways.[2–4] Importantly, genetics of AD support a critical role for glia and neuroinflammation in AD,[5–7] whereas clinical studies suggest prevalent presence of pathologies affecting the brain's white matter.[8,9] Overall, vascular health, gliopathy, and neuroinflammation appear to contribute to disease progression.[2,10,11] Consequently, a better characterization of primary immuno-vascular dysregulations and the related changes in synaptic plasticity[12] in AD emerge as critical areas of research. With improved technologies, noninvasive biomarkers of these molecular pathologies are becoming increasingly feasible to quantify. This presents a unique opportunity for investigation of alternative or synergistic pathological processes to the classical proteinopathies (Aβ and tau) and their association with established measures of neurodegeneration and cognitive impairment.

Numerous studies have begun to investigate plasma biomarkers in AD; however, most have concentrated on detecting AD proteinopathies or neuronal injury.[13,14] This project aimed to contribute to the characterization of plasma biomarkers in AD, across a wider spectrum of pathways, with a particular interest in extending knowledge of early-onset AD (EOAD). Clinical observations suggest a more aggressive disease course in EOAD,[15] although specific molecular differences between sporadic EOAD and late-onset AD (LOAD) remain to be elucidated. To this end, we selected a panel of validated, commercially available ultrasensitive immunoassays for quantification of plasma markers in both AD groups.

We aimed to capture inflammation that has been well documented in AD states[16–18] (Table 1). Our analytes relate to inflammasome formation (IL1β)[19–22] and systemic inflammation (IL-6, TNFα, IP-10, IL-17A).[16,18,23–26] To capture neuroinflammation, we quantified a marker of astrocytic activation (GFAP).[27,28] Neurofilament light (NfL) change was quantified as a measure of neuronal axonal injury.[13,29] Moreover, we selected biomarkers of perturbation in neural, glial, and vascular plasticity and homeostasis (BDNF, VEGF, and TGFβ). BDNF can be secreted by all cell types and has been well described as a regulator of synaptic plasticity.[30,31] VEGF is involved in angiogenic growth and blood-brain barrier (BBB) permeability.[32,33] Similarly, TGFβ is a major regulator of neuroinflammation and vascular homeostasis, with critical roles at the BBB junction,[34,35] including astrocytic activation[36,37] and induction of important changes to neuronal extracellular matrix and hyperexcitability.[36–38]

This study takes an initial step toward demonstration of the utility of the selected plasma biomarkers in EOAD and LOAD. To our knowledge, only one prior study has looked at

IL-6, TNFα, and IL1β in very small groups of EOAD and LOAD. Therefore, very little is known about levels of these biomarkers in EOAD and comparison with LOAD or typically aging normal controls. Applying the selected proteomic panel, we evaluated individual biomarkers in addition to combined effects of related biomarkers in 33 EOAD and 30 LOAD subjects compared with 36 typically aging adults (Fig. 1). In addition, we examined the relationship between our markers and clinical variables of interest, such as neurobehavioral deficits, neuroimaging, and demographics.

2 | METHODS

2.1 | Study participants

We performed a cross-sectional sampling from ongoing longitudinal cohorts at the UCSF Memory and Aging Center of participants with a diagnosis of AD (late and early-onset) as well as healthy interview, culminating in a consensus diagnostic conference determining their clinical diagnoses of normal, MCI, or dementia due to probable AD (National Institute on Aging and Alzheimer's Association).[39] We required a positive amyloid positron emission tomography (PET) scan within a year of blood draw (n = 14) for subjects meeting only "possible AD." For EOAD classification, onset of cognitive symptoms had to be before age 65, with a positive amyloid PET within one year of the blood draw. Controls were functionally normal (Clinical Dementia Rating [CDR] = 0 via study partner interview) with negative amyloid PET scans as available (n = 18), and all demonstrated no significant white matter hyperintensity (WMH; Fazekas 0–1).[40] All study participants provided informed consent, and the study protocols were approved by the UCSF Committee on Human Research. Research was performed in accordance with the Code of Ethics of the World Medical Association.

2.2 | Structural neuroimaging (MRI)

Volumetric MPRAGE sequences were used to acquire T1-weighted images, and fluid attenuated inversion recovery magnetic resonance imaging was acquired for WMH quantification. Tissue segmentation was performed using SPM12 (Wellcome Trust Center for Neuroimaging, London, UK, http://www.fil.ion.ucl.ac.uk/spm) unified segmentation.[41] A group template was generated using the large deformation diffeomorphic metric mapping framework.[42] Native subject spaces were normalized, modulated, and smoothed in the group template. The total intracranial volume was calculated using tissue segmentations based on.[43] Linear and nonlinear transformations between the group template space and International Consortium of Brain Mapping were applied. WMH quantification was estimated by WMH segmentation[44,45] using fluid attenuated inversion recovery and T1-weighted images.

2.3 | Molecular neuroimaging (amyloid PET)

Aβ status was derived from PET with either 11C-Pittsburg compound B (injected dose: ~15 mCi; 57% of participants) or 18F-florbetapir (injected dose: ~10 mCi; 43%). To determine amyloid-PET positivity, we applied standardized uptake value ratio thresholds derived from previous publications using identical preprocessing pipelines (1.21 for 11C-Pittsburg compound B,[46] 1.11 for florbetapir[47]). Both thresholds have also been autopsy-validated.

[46]–[49] Note that for florbetapir, acquisition and preprocessing followed ADNI procedures Supplementary Material.

2.4 | Plasma protein quantification

Venous blood was collected in EDTA-containing tubes, and plasma samples were stored in 0.25 mL aliquots at −80°C. Samples (1 thawing only) were gradually brought to room temperature for analyses. The ultrasensitive HD1 analyzer by Quanterix (Lexington, MA), which employs an automated single molecule array (Simoa) principle, was used for quantification of proteins. IL-10, IL-6, and TNFα were measured via multiplex, whereas all other analytes were measured using single analyte assays (GFAP, NfL, BDNF, VEGF, TGFβ, IP-10, and IL1β). All analyses were performed in duplicate, according to manufacturer's published protocols. Samples with coefficients of variance >20% were excluded from analyses. Final data were also examined for extreme outliers, and samples with >50x the upper interquartile range were also excluded (n = 1 on BDNF).

2.5 | Neuropsychological evaluation

All tests have been described in detail elsewhere[50] and were selected to be sensitive to age-related neurologic disease and domains particularly affected in AD.[50] Specifically, we examined *global cognition* (Mini–Mental State Examination; n = 84), *executive functions* (modified Trail Making Test, Digit Span Backward, Stroop Inhibition, Lexical fluency, Delis–Kaplan Executive Function System Design Fluency: Condition 1; n = 58), *episodic memory* (modified Benson Figure Delayed Recall; n = 74), *processing speed* (Stroop Color Naming; n = 68), *spatial functioning* (modified Benson Figure Copy and Visual Object and Space Perception Spatial Discrimination; n = 69), and *semantic processing* (Semantic Fluency, abbreviated Boston Naming Test; n = 76). All raw scores were standardized to the study sample, and scores were averaged for domains with multiple measures; composites were only calculated for individuals with available data for each test per domain. CDR and CDR sum of boxes (CDRsob) were completed on all participants via study partner interviews.

2.6 | Statistical analyses

All plasma markers were log transformed to normalize the distributions. Although age is inherently different between AD groups, to emphasize disease-related differences over age-associated differences, age at blood draw was included in all analyses as a covariate. However, we also provide analyses not adjusting for age for readers interested in the combined effects of age and disease while highlighting differences between age-adjusted and unadjusted models. We used analysis of variance (ANOVA), and ANCOVAs (controlling for age) with post hoc Tukey honestly significant difference to determine pairwise differences. To limit multiple comparisons, we conducted principal component analyses (PCAs) estimating covariance matrices (orthogonal component estimates); missing values were estimated via unstructured restricted maximum likelihood to optimize bias correction (e.g., over maximum likelihood). Only analytes with reliable measurements (i.e., coefficient of variance <20%) on >75% of the sample were included in the PCA, which excluded IL-17A and IL1β (i.e., 9 analytes in final PCA). PCA extraction with (n = 99) and without (n = 41) restricted maximum likelihood–based imputation were conducted. Given

the exact pattern of results held regardless of imputation, all reported PCA analyses were conducted on extracted values with imputation. Using the extracted PCA components, we examined group differences via ANCOVA with post hoc Tukey honestly significant difference. We also conducted multivariable regression models examining associations between each PCA component and demographic and clinical variables of interest. Finally, for the two factors (IL1β and IL17A) that were not included in the final PCA components, we report on associations with demographics, neurobehavioral, and neuroimaging outcomes, adjusting for age.

3 | RESULTS

3.1 | Demographics

Participant characteristics are summarized in Table 2. As expected, the mean age for patients with LOAD and controls was significantly higher than EOAD ($P < .001$). Consequently, in light of age being itself a risk for neurodegeneration and pathological molecular changes, we controlled for age in all our analyses to capture effects exceeding those associated with aging. There were no significant differences with respect to sex and educational attainment between groups. Measures of cognitive function and functional independence were as expected different between AD groups and controls; however, this did not differ between EOAD and LOAD (Table 2).

3.2 | Molecular marker group differences

Markers of astrocytic and neuronal degeneration and neuronal plasticity (GFAP, NfL, BDNF) demonstrated significant group differences. Pairwise comparisons demonstrated that GFAP was higher in both AD groups in comparison with controls, with a larger effect size in EOAD (Cohen's d = 1.6, $P = .006$) than LOAD (Cohen's d = 0.98, $P = .003$). When controlling for age, NfL was only significantly higher in EOAD compared with the control group (Cohen's d = 1.3, $P = .005$), with a smaller effect size in LOAD that trended but did not reach significance (Cohen's d = 0.57, $P = .08$). BDNF was highest in LOAD compared with controls (Cohen's d 0.78, $P = .03$). VEGF was also highest in LOAD; however, the difference with controls did not reach significance (Cohen's d = 0.62, $P = .07$). No individual inflammatory marker (IL1β, IL-6, IP-10, TNFa, IL-10, IL17A), nor TGFβ, significantly differed across groups. Group comparisons without adjustment for age overall demonstrated similar findings (Fig. 3, Supplementary Fig. 1; Table 4). The main differences found were that NfL in EOAD was no longer significantly higher than controls, whereas LOAD was higher than controls (Cohen's d = 0.79, $P = .006$). Other differences included VEGF that was found to be higher in EOAD in comparison with controls (Cohen's d = 0.064, $P = .03$ for VEGF) and IP-10 that demonstrated a strong age-associated effect, with significantly lower levels in EOAD in comparison with both controls (Cohen's d = −1.0, $P = .001$) and LOAD (Cohen's d = −1.5, $P < .001$) (Figs. 2–4, Supplementary Fig. 1, Supplementary Fig. 2; Tables 3 and 4).

PCA evidenced 3 primary components explaining 69.3% of the variance, with subsequent components contributing substantially less (<10%) (Fig. 2; Table 3). Component 1: "inflammatory factor" (30.1% variance), demonstrated highest loadings from the

inflammatory markers (IP-10, IL-10, IL-6, TNFα). Component 2: "trophic factor" (22.7% variance), demonstrated highest loadings from trophic signaling proteins (BDNF, VEGF, TGFβ). Component 3: "degenerative factor" (16.5% variance), demonstrated highest loadings from neuronal and astrocytic structural proteins (NfL, GFAP).

Trophic factor demonstrated the largest difference across groups, with both EOAD and LOAD groups demonstrating elevated levels compared with controls, in both age-adjusted and nonadjusted models (Fig. 4, Supplementary Fig. 2; Table 4). The degenerative factor also differed across groups, with EOAD individuals exhibiting the highest levels compared with controls. In contrast, the inflammatory factor did not differ across groups. The most notable difference in group comparisons without adjustment for age was again pertaining to inflammation with the inflammatory factor being lower in EOAD in comparison with LOAD (Cohen's d = −0.84, P = .004).

3.3 | Molecular marker associations with demographics and neurobehavioral measures

3.3.1 | Demographics—Combining groups, only the inflammatory factor was significantly associated with age (r = 0.38, P < .001; Trophic factor r = −0.01, P = .92; Degenerative Factor r = −0.07, P = .51). Females had higher trophic factor values (t = 2.1, P = .04) but did not differ on the other factors (Inflammatory factor t = −1.3, P = .20; Degenerative factor t = 1.4, P = .16). None of the factors were significantly associated with educational attainment (r range = −0.10 to 0.03, P range = 0.33 to 0.76) (Fig. 5, Supplementary Fig. 2; Tables 5 and 6).

3.3.2 | Functional impairment—Covarying for age across all groups, higher trophic (β = 0.38, P < .001) and inflammatory factors (β = 0.24, P = .03) were associated with greater functional impairment (CDRsob). Interestingly, the degenerative factor was not statistically associated with severity of impairment (β = 0.11, P = .28); however, there was a significant group*degenerative factor interaction, such that higher degenerative factor levels were associated with disproportionately greater functional impairment in the EOAD group [F (2) = 4.1, P = .02] (Table 5).

3.3.3 | Cognitive performance—Higher trophic factor was associated with worse neuropsychological performances across all domains examined, including lower global cognition (Mini–Mental State Examination; β = −0.60, P < .001), episodic memory (β = −0.59, P < .001), executive function (β = −0.59, P < .001), visuospatial functioning (β = −0.33, P = .006), semantic processing (β = −0.47, P = .002), and overall slower processing speed (β = −0.38, P < .001), all covarying for age. There were no significant group*trophic factor interactions on cognition (Ps > 0.12) (Table 5).

Higher values of the degenerative factor were associated with diminished spatial processing abilities (β = −0.34, P = .004), with a similar nonsignificant trend for processing speed (β = −0.21, P = .08), executive functions (β = −0.24, P = .051), and semantic processing (β = −0.27, P = .08). The degenerative factor was not statistically associated with global cognition (Mini–Mental State Examination; β = −0.05, P = .63) or episodic memory (β = −0.18, P = .10), and there were no significant group*degenerative factor interactions on cognition (Ps > 0.12).

The inflammation factor was not associated with performance in any cognitive domain (Ps > 0.22), nor were there any significant group*inflammatory factor interactions on cognition (Ps > 0.10).

3.4 | Molecular marker associations with neuroimaging

Combining groups, and adjusting for age and total intracranial volume, elevated trophic factor levels were associated with smaller total gray matter volume (GMV; $\beta = -0.49$, $P < .001$). The degenerative factor did not reach significance with GMV ($\beta = -0.16$, $P = .19$), and the inflammatory factor showed an even smaller, nonsignificant effect ($\beta = -0.08$, $P = .55$) (Fig. 5; Tables 5 and 6).

Using Fazekas grading for WMH,[40] we identified AD individuals (EOAD and LOAD) with high WMH burden (Fazekas   2) and conducted a 3-level group analysis (control vs. AD without WMH vs. AD with WMH) across the three components. The degenerative [$F_{(3, 95)} = 3.2$, $P = .03$] and trophic [$F_{(3, 95)} = 6.6$, $P < .001$] factors demonstrated significant group differences. Specifically, AD individuals with high WMH demonstrated higher degenerative levels compared with controls ($P = .003$), whereas AD individuals regardless of WMH showed higher trophic factor (Ps < 0.01) (Fig. 5, Table 6). The inflammatory factor did not statistically differ across groups ($P > .22$). Group comparisons without adjustment for age demonstrated similar results (Supplementary Fig. 2).

Furthermore, in light of the role of astrogliosis in WMH, we specifically investigated the association of GFAP with WMH and found a significant linear association ($\beta = 0.27$, $P = .03$). The biomarker of axonal injury, NfL, was also significantly associated with volumes of WMH ($\beta = 0.35$, $P = .004$).

3.5 | Post hoc analyses: IL1β and IL17A

3.5.1 | Demographics—The cytokines excluded from PCA-based factor constructions (IL1β and IL17A) because of smaller sample sizes resulting from elimination of values with lower reliability (high coefficients of variance) were analyzed separately, so as to inform future studies with larger sample sizes. Regarding demographics, IL1β and IL17A were not associated with age (IL1β $\rho = 0.09$, $P = .45$; IL17A $\rho = 0.10$, $P = .46$) or education (IL1β $\rho = 0.14$, $P = .33$; IL17A $\rho = 0.04$, $P = .73$). Females demonstrated lower mean levels of IL1β ($\beta = -0.31$, $P = .01$), but no difference was noted with respect to IL17A ($\beta = -0.06$, $P = .67$).

3.5.2 | Cognitive function—Higher IL1β was also associated with greater functional impairment (CDRsob; $\beta = 0.34$, $P = .009$), but no association was noted with IL17A ($\beta = 0.04$, $P = .81$), within age-adjusted models. IL1β and IL17A were not associated with performance across any of the cognitive domains (Ps > 0.17) and did not interact with group status to predict cognition or functioning ($P > .3$).

3.5.3 | Neuroimaging—IL1β ($\beta = -0.19$, $P = .18$) and IL17A ($\beta = -0.33$, $P = .06$) were not associated with total GMV. Interestingly when examining WMH, IL1β showed differential elevations across groups [$F_{(3, 62)} = 2.3$, $P = .09$], such that AD individuals

without WMH had the highest IL1β concentrations compared with those with WMH and controls (P = .02). IL17A did not differ across WMH groups (P > .22).

4 | DISCUSSION

We used plasma-based proteomic markers to evaluate differential molecular pathways in EOAD and LOAD. Specifically, we found that plasma levels of markers of cellular plasticity were strongly associated with functional, cognitive, and imaging outcomes and elevated in both AD groups. Structural proteins, reflecting neural and glial degenerative states, were particularly elevated in EOAD, including a significant association with functional status in EOAD, with weaker associations to cognitive and imaging outcomes. Interestingly, proteins associated with systemic inflammation did not differ across groups and were associated with age, white matter injury, and functional impairment, but not other neurobehavioral outcomes.

We observed elevation in a marker of astrocytic activation and degeneration, GFAP, in EOAD and to a lesser extent LOAD. This finding confirms the previously known affectation of astrocytes in AD[51–53] and suggests that GFAP could be a technically reliable and useful biomarker of interest in future observational and interventional studies of AD. In fact, a recent pathological study in AD demonstrated a critical impairment in the glymphatic system, which is heavily dependent on the connected network of astrocytes.[54] Detection of GFAP in plasma lacks spatial information; therefore, all that can be said based on our data is that there seems to be a potential activation and/or degeneration of astrocytes in EOAD and LOAD. Astrocytic pathology in AD has important therapeutic implications. Over the past two decades, brain glia, including astrocytes, have emerged as important cellular components of brain health, with notable dysfunction in neurodegenerative disease. Although astrocytes do not directly communicate by chemical synapses, they have the ability to critically alter the neuronal environment through secretion of several gliotransmitters and trophic factors, and by regulating the propagation of calcium signaling/ waves and neurotransmitter availability.[55] Our data further corroborate and extend these studies as one of the first to demonstrate that a marker of astrocytic activation and degeneration is elevated in plasma of subjects with EOAD and LOAD and that levels are associated with degree of white matter injury (WMH).

Similarly, NfL, a marker of neuronal axonal injury, and the degenerative composite (GFAP and NfL) demonstrated elevations in AD. NfL has gained much attention as an indicator of axonal injury across neurologic and neurodegenerative diseases, including LOAD.[56] Most interesting is our finding that structural markers of degeneration (degenerative factor) was elevated in EOAD and particularly associated with functioning. Together, these findings support the notion that EOAD appears to be a particularly aggressive form of AD with rates of clinical progression typically exceeding those in LOAD[57].

As a whole, the trophic factors demonstrated strong associations with cognition, functional impairment, and GMV atrophy. These factors can be secreted peripherally and centrally, with especially high levels of secretion by glial support cells.[58] The combination of GFAP and trophic factor elevations among AD groups underscores the loss of homeostasis evident

in states of neurodegenerative disease. The observed alterations in growth factors (i.e., BDNF, VEGF) may represent compensatory remodeling of both neural and vascular systems in response to degeneration as demonstrated *in vitro* and in animal models of disease.[59,60]

Given that these markers are measured in plasma, with the exception of NfL and GFAP, their source is likely to be peripheral. However, their strong association with brain-specific outcomes suggests that they may be good markers of central nevous system (CNS) disease. There are several possibilities explaining their association: (1) due to active CNS disease and through progressive degeneration of the BBB, peripheral levels are reflecting CNS pathophysiology (e.g., synaptic plasticity), (2) they are reflecting shared molecular mechanisms across peripheral and CNS compartments (e.g., cellular senescence), and/or (3) they are not directly biologically associated with CNS molecular milieu but instead reflect parallel age-related biological vulnerabilities. Nonetheless, the state of dysregulated homeostasis represented by trophic factors appears to be consistent indicators of clinical disease status, even as measured in the periphery.

The inflammatory factors, especially IP-10, demonstrated age effects, in congruence with the large body of work on immune senescence[61] and development of a potentially reactive state of chronic inflammation, where proinflammatory cytokines secreted by degenerating parenchymal and immune cells increase in the setting of a senescent immune system.[62] However, when adjusting for age, they were neither significantly different among groups nor did they demonstrate associations with clinically meaningful outcomes. Our approach was limited in that we only measured plasma markers that may not adequately reflect neuroinflammation.[18] In addition inflammation may have a stage-dependent importance, with a larger effect at time of conversion to MCI.[63] It is also plausible that inflammation is a critical component of the risk that aging represents for the development of neurodegenerative disorders, and once in disease states, the role of inflammation may relatively lessen. Therefore, the absence of young controls in this study and the stage of disease included may be limiting the variance needed for capture contributions from systemic inflammation.

We note that these biomarkers have previously been studied in model systems and individually in human studies and that the novelty of our work lies in their comparison between EOAD and LOAD groups included in our study. Although our study represents a novel approach to the investigation of noncanonical pathologies in AD, across early-and late-onset disease, we are limited to detecting only relatively larger effects given our sample sizes. As in any studies, replication of findings in an independent cohort would be valuable. In addition, inclusion of younger controls and study of markers in cerebrospinal fluid would be of great value. An additional limitation of the present study is the quantification of these markers in plasma without a measure of BBB impairment. Plasma levels may represent the compounded outcomes of glial activation (GFAP), cellular degeneration (NfL and GFAP), in addition to possible changes in BBB permeability.

The biomarkers we quantified are likely to capture inter-related molecular pathways involved in inflammation, astrocytic activation, BBB, and neuronal dysfunction and degeneration. We could only demonstrate their covariance via statistical methods such as

principle component analyses and did not have the data for pathway analyses. There are complex, likely stage-dependent, relationships between systemic and CNS inflammation, astrocytic activation, and cellular (glial and neuronal) degeneration in AD. In this cross-sectional study, we cannot investigate mechanisms. We simply report on concentration differences and their association with clinically meaningful outcomes. Future longitudinal studies characterizing these plasma markers in EOAD and LOAD from early symptoms to dementia will provide invaluable information regarding their temporal sequencing, risk thresholds, and association with gold-standard A/T/N biomarkers[39] and clinical symptoms. [64] Such studies will require large collaborative efforts, such as ADNI[65] and LEADS (longitudinal early-onset Alzheimer's disease study) consortia. Ultimately, observational modelling has its limits and quantification of markers in cohorts with clinical interventions are critically needed to determine the nature of the relation (causal vs. consequential) of these novel biomarkers to AD pathogenesis. Only through acknowledgement and measurement of the *host* of affected molecular pathways will we be able to address the heterogeneity of AD and begin to unlock the important underlying pathophysiological changes needed for the development of novel therapeutics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

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RESEARCH IN CONTEXT

1. Systematic review: The authors reviewed the available scientific literature on PubMed for articles examining proteomic biomarkers in Alzheimer's disease (AD). Although abundant studies examine the role of amyloid and tau in AD, relatively fewer have explored alternative molecular markers in clinical studies. In this study, we investigated the relationship between plasma markers in early versus late age of onset sporadic AD.

2. Interpretation: Our findings suggest that plasma-based proteomic markers reflecting astrocytic activation, neuronal degeneration, and cellular plasticity differ across groups. Markers of plasticity were associated with functional decline. Inflammatory proteins only correlated with age in this study.

3. Future directions: cerebrospinal fluid–based quantification of markers that are also secreted by cells outside of the CNS (e.g., BDNF and VEGF) will provide insight into brain-systemic compartment differences in AD. Expansion to an unbiased quantification of plasma proteins could further uncover discrete subgroups within the heterogeneous landscape of AD pathophysiology.
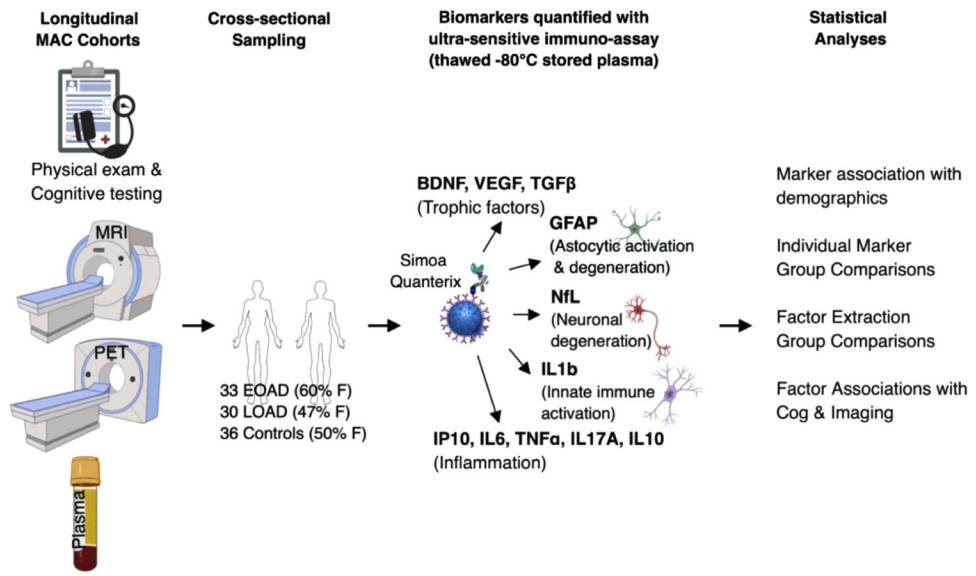
FIGURE 1.
Experimental design.
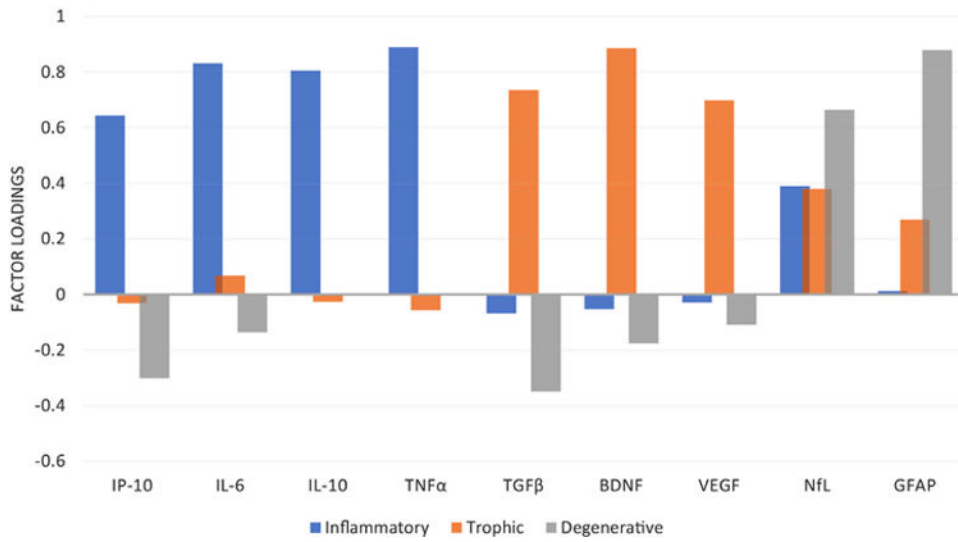
FIGURE 2.
Factor loadings by plasma biomarker for three main principle components.
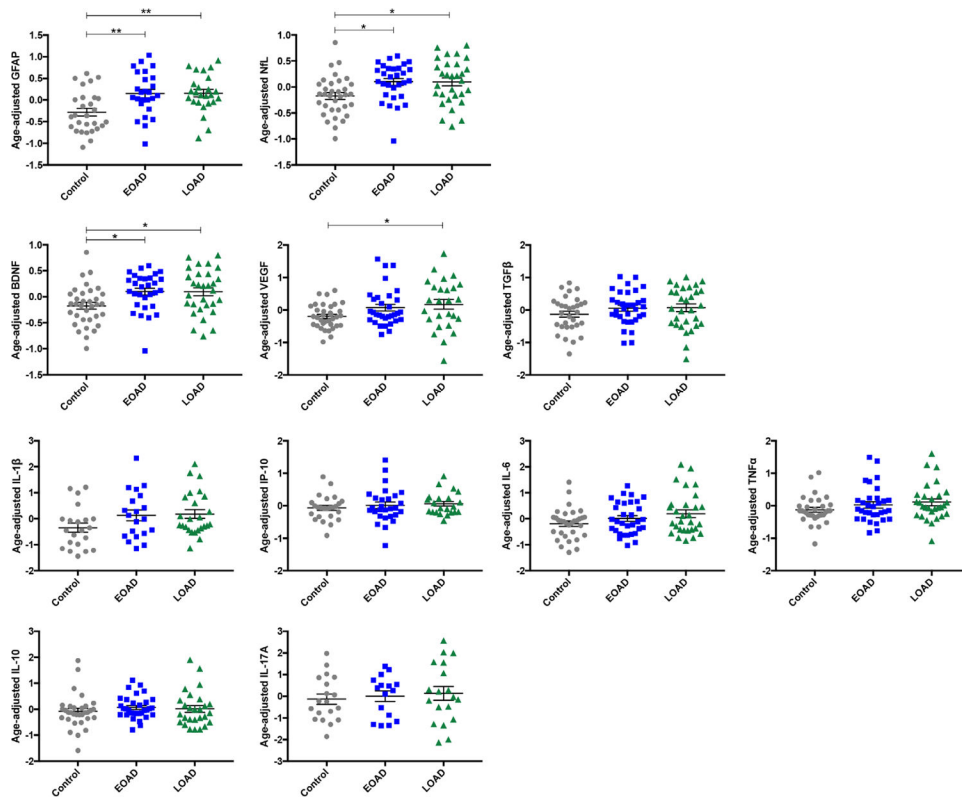
FIGURE 3.

Comparison of plasma biomarkers across control, EOAD, and LOAD groups. Comparison of plasma biomarkers across groups (controls, EOAD, LOAD). The values depicted were log-transformed to normalize distributions. Stars depict significance levels from age-adjusted ANCOVA models with post hoc Tukey HSD test: ** .01; * .05. Abbreviations: EOAD, early-onset Alzheimer's disease; LOAD, late-onset Alzheimer's disease; HSD, honestly significant difference.
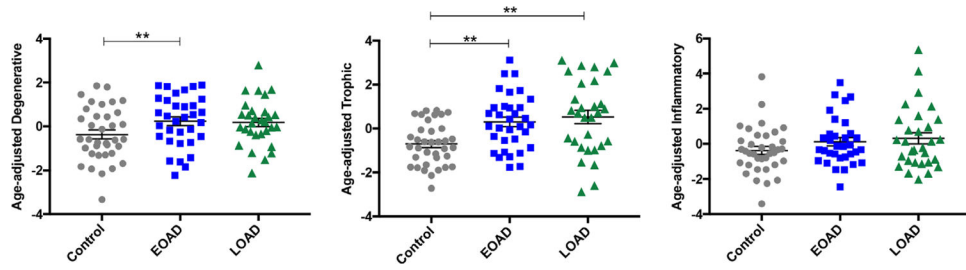
FIGURE 4.
Principle components compared across control, EOAD and LOAD groups. Comparison of principle components (degenerative, trophic, and inflammatory factors) across groups. Stars depict significance levels from age-adjusted ANCOVA models with post hoc Tukey HSD test: ** .01; * .05. Abbreviations: EOAD, early-onset Alzheimer's disease; LOAD, late-onset Alzheimer's disease; HSD, honestly significant difference.
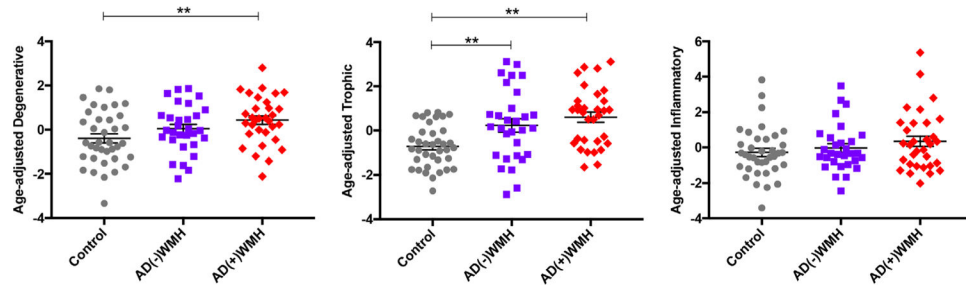
FIGURE 5.
Principle components compared across groups based on extent of WMH. Comparison of principle components (degenerative, trophic, and inflammatory factors) across groups divided based on extent of white matter injury. Stars depict significance levels from age-adjusted ANCOVA models with post-hoc Tukey HSD test: ** .01; * .05. Abbreviations: AD−WMH, Alzheimer's disease without white matter hyperintensity (Fazekas score 0–1); AD+WMH, Alzheimer's disease with white matter hyperintensity (Fazekas score 2–3); HSD, honestly significant difference.

TABLE 1

List of plasma proteomic biomarkers included in this study

Acronym	Name	Function
NfL	Neurofilament light polypeptide	Neuronal axonal cytostructural protein
GFAP	Glial fibrillary acidic protein	Astrocytic cytostructural protein, unregulated at activation
BDHF	Brain-derived neurotrophic factor	Secreted by a variety of cell types, including glia, involved in synaptic plasticity and neuronal functional homeostasis
VEGF	Vascular endothelial growth factor	Secreted by glia, neurons, and endothelialcells involved in angiogenesis and barrier permeability
TGFβ	Transforming growth factor β	Capturing TGFβ (isoforms 1–3), cytokines secreted by many cell types, including macrophages with pleiotropic effects
IL1β	Interleukin 1 β	Cytokine involved in inflammasome formation and innate immunity
IP-10	Interferon gamma-induced protein 10	Secreted in response to IFN-Y by endothelia, monocytes, and other cell types and associated with barrier permeability
IL-6	Interleukin 6	Acts as both a proinflammatory cytokine and an antiinflammatory myokine
TNFα	Tumor necrosis factor α	Cytokine involved in systemic inflammation
IL-10	Interleukin 10	Antiinflammatory cytokine that can block NF-κB activity
IL-17A	Interleukin 17 A	Proinflammatory cytokine, produced by activated T-cells among other cell types and can stimulate IL-6 production

TABLE 2

Summary of demographic and basic clinical data for all groups

Diagnosis	Controls, n = 33		EOAD, n = 33		LOAD, n = 30		Total, n = 99
	Mean	SD	Mean	SD	Mean	SD	P value
Age, years	72	8.40	61	6.20	79	4.70	<.0001
Sex, F/M, %	0.50		0.60		0.47		.5
Education, years	17.60	2	16.8	2.60	16.50	3.70	.35
MMSE	29	1	23	3.7	25	3.7	<.0001
CDRTot	0	0	0.80	0.40	0.80	0.60	<.0001
CDRBox	0	0	4.70	1.90	4.40	3.30	<.0001
Memory	1.10	0.43	−0.67	0.50	−0.53	0.82	.001
Executive functions	0.88	0.49	−0.64	0.51	−0.25	0.48	<.001
Processing speed	0.81	0.68	−0.80	0.68	−0.35	0.76	<.001
Semantic processing	0.74	0.35	−0.45	0.86	−0.56	0.75	.001
Spatial processing	0.53	0.19	−0.45	1.10	−0.15	0.97	.0002

NOTE. Cognitive scores reflect unadjusted z-scores calculated via sample-based standardized composites. Abbreviations: EOAD, early-onset Alzheimer's disease; LOAD, late-onset Alzheimer's disease; cSVD, cerebral small vessel disease; ANOVA, analysis of variance; M, male; F, female; MMSE, Mini–Mental State Examination score; CDRTot, clinical dementia rating score total; CDRBox, CDR box score; SD, standard deviation.

TABLE 3

Component eigenvalues with proportion of variance explained across the sample

Number	Eigenvalue	% Variance	Cumulative % variance
1	2.7067	30.074	30.074
2	2.0402	22.669	52.743
3	1.4874	16.527	69.27
4	0.8507	9.453	78.723
5	0.6583	7.314	86.037
6	0.4588	5.098	91.135
7	0.3594	3.993	95.128
8	0.2409	2.677	97.805
9	0.1976	2.195	100

TABLE 4

ANCOVA and ANOVA models comparing individual biomarkers across groups

	ANCOVA Models						ANOVA Models					
	Model				**Post-hoc (Tukey HSD)**		**Model**				**Post-hoc (Tukey HSD)**	
	Adj. R^2	F (df)	Partial η^2	P value	Cohen's d (95% CI)	P value	Adj. R^2	F (df)	η^2	P value	Cohen's d (95% CI)	P value
Markers of neuronal and astrocytic structural change and degeneration												
NfL												
Model	0.16	6.8 (3, 93)		.0003			0.08	5 (2, 93)		.009		
Age			0.10	.003					–	–		
Group			0.14	.001					0.10	.009		
EOAD>Control					**1.3 (0.47, 2.1)**	**.005**					0.30 (−0.19, 0.80)	.45
LOAD>Control					0.57 (0.05, 1.1)	.08					**0.79 (0.29, 1.3)**	**.006**
EOAD>Control					0.70 (−0.23, 1.6)	.3					−0.49 (−0.10, 0.03)	.15
GFAP												
Model	0.22	8.3 (3, 78)		<.001			0.21	11.1 (2, 78)		<.001		
Age			0.03	.13					–	–		
Group			0.25	<.001					0.23	<.001		
EOAD>Control					**1.6 (0.74, 2.4)**	**.006**					**1.1 (0.55, 1.7)**	**.0003**
LOAD>Control					**0.98 (0.40, 1.6)**	**.003**					**1.1 (0.51, 1.6)**	**.0005**
EOAD>LOAD					0.60 (−0.33, 1.5)	.42					0.03 (−0.53, 0.58)	.99
Markers of cellular plasticity												
BDNF												
Model	0.09	3.5 (3, 82)		.02			0.09	5.0 (2, 82)		.009		
Age			0.01	.41					–	–		
Group			0.12	.007					0.11	.009		
EOAD>Control					0.81 (0.02, 1.6)	.11					0.56 (0.05, 1.1)	.08
LOAD>Control					**0.78 (0.19, 1.4)**	**.03**					**0.84 (0.28 1.4)**	**.01**
EOAD>LOAD					0.03 (−0.91, 0.56)	.99					−0.29 (−0.85, 0.27)	.57
VEGF												
Model	0.06	2.9 (3, 92)		.04			0.07	4.3 (2, 92)		.02		
Age			0.0002	.89					–	–		

	ANCOVA Models						ANOVA Models					
	Model				**Post-hoc (Tukey HSD)**		**Model**				**Post-hoc (Tukey HSD)**	
	Adj. R²	F (df)	Partial η²	P value	Cohen's d (95% CI)	P value	Adj. R²	F (df)	η²	P value	Cohen's d (95% CI)	P value
Group			0.08	.03					0.09	.02		
EOAD>Control					0.59 (−0.14, 1.3)	.25					**0.64 (0.15, 1.1)**	**.03**
LOAD>Control					0.62 (0.07, 1.2)	.07					0.61 (0.09, 1.1)	.06
EOAD>LOAD					−0.02 (−0.32, 0.87)	.99					0.03 (−0.50, 55)	.99
TGFβ												
Model	0.003	1.09 (3, 93)		.36			0.006	1.3 (2, 93)		.29		
Age			0.008	.39					–	–		
Group			0.03	.24					0.03	.29		
EOAD>Control					0.49 (−03, 1.2)	.40					0.24 (−0.25, 0.73)	.59
LOAD>Control					0.32 (−0.21, 0.86)	.46					0.40 (−0.10, 0.91)	.26
EOAD>LOAD					0.16 (−0.7, 1.0)	.93					0.16 (−0.34, 0.66)	.80
Markers of inflammation (cytokines)												
IL1β												
Model	0.04	1.9 (3, 65)		.14			0.04	2.4 (2, 65)		.10		
Age			0.01	.37					–	–		
Group			0.08	.07					0.07	.10		
EOAD>Control					0.83 (−0.14, 1.8)	.21					0.50 (−0.12, 1.1)	.26
LOAD>Control					0.55 (−0.06, 1.2)	.17					0.63 (0.03, 1.2)	.10
EOAD>LOAD					0.28 (−0.79, 1.3)	.87					−0.13 (−0.72, 0.46)	.90
IP-10												
Model	0.38	16.0 (3, 74)		<.001			0.28	15.1 (2, 74)		<.001		
Age			0.007	.65					–	–		
Group			0.11	.0007					0.30	<.001		
EOAD>Control					−0.16 (−1.03, 0.71)	.93					**−1.0 (−1.6, −0.44)**	**.001**
LOAD>Control					0.27 (−0.33, 0.86)	.65					0.53 (−0.06, 1.1)	.18
EOAD>LOAD					−0.11 (−1.13, 0.91)	.98					**−1.5 (−2.1, −0.91)**	**<.001**
IL-6												
Model	0.11	4.9 (3, 92)		.004			0.10	6.3 (2, 92)		.003		
Age			0.02	.16					–	–		

	ANCOVA Models						ANOVA Models					
	Model				**Post-hoc (Tukey HSD)**		**Model**				**Post-hoc (Tukey HSD)**	
	Adj. R²	F (df)	Partial η²	P value	Cohen's d (95% CI)	P value	Adj. R²	F (df)	η²	P value	Cohen's d (95% CI)	P value
Group			0.03	.22					0.12	.003		
EOAD>Control					0.09 (−0.66, 85)	.97					−0.32 (−0.81, 0.17)	.40
LOAD>Control					0.47 (−07, 1.0)	.20					0.59 (0.07, 1.1)	.06
EOAD>LOAD					−0.38 (−0.85, 0.66)	.69					−0.91 (−1.4, 0.38)	.002
TNFα												
Model	0.008	1.3 (3, 92)		.30			0.01	1.4 (2, 92)		.24		
Age			0.01	.35					–	–		
Group			0.02	.47					0.03	.24		
EOAD>Control					0.25 (−0.50, 1.0)	.79					−0.02 (−0.51, 0.46)	.99
LOAD>Control					0.29 (−0.24, 0.83)	.53					0.37 (−0.14, 0.88)	.32
EOAD>LOAD					−0.03 (−0 94, 0.87)	.79					−0.40 (−0.90, 0.12)	.28
IL-10												
Model	0.02	1.8 (3, 90)		.16			−0.01	0.60 (2, 90)		.55		
Age			0.04	.047					–	–		
Group			0.02	.33					0.01	.54		
EOAD>Control					0.58 (−0.19, 1.3)	.31					−0.03 (−0.46, 0.51)	.99
LOAD>Control					0.08 (−0.46, .61)	.96					0.24 (−0.28, 0.75)	.63
EOAD>LOAD					0.50 (−0.41, 1.4)	.53					−0.26 (−0.78, 0.25)	.57
IL-17A												
Model	0.04	0.27 (3, 53)		.85			−0.03	0.32 (2, 53)		.73		
Age			0.003	.69					–	–		
Group			0.001	.96					0.01	.73		
EOAD>Control					0.02 (−0.93, 97)	.99					−0.12 (−0.78, 0.53)	.93
LOAD>Control					0.10 (−0.59, 0.78)	.96					0.15 (−0.49, 0.79)	.89
EOAD>LOAD					0.08 (−1.1, 1.2)	.99					−028 (−95, 0.40)	.70
Degenerative, inflammatory, and trophic PCA analyses												
Degenerative factor												
Model	0.06	3.2 (3, 98)		.03			0.06	4.3 (2, 98)		.02		
Age			0.01	.30					–	–		

	ANCOVA Models						ANOVA Models					
	Model				Post-hoc (Tukey HSD)		Model				Post-hoc (Tukey HSD)	
	Adj. R²	F (df)	Partial η²	P value	Cohen's d (95% CI)	P value	Adj. R²	F (df)	η²	P value	Cohen's d (95% CI)	P value
Group			0.09	.01					0.08	.02		
EOAD>Control					**0.95 (0.22, 1.7)**	**.03**					**0.66 (0.18, 1.1)**	**.02**
LOAD>Control					0.44 (−0.07, 0.96)	.21					0.53 (0.04, 1.0)	.09
EOAD>LOAD					0.51 (−0.36, 1.4)	.49					0.13 (−0.36, 0.63)	.86
Trophic factor												
Model	0.14	65 (3, 98)		.0005			0.14	9.3 (2, 98)		.0002		
Age			0.01	.31					–	–		
Group			0.17	.0001					0.16	.002		
EOAD>Control					**1.1 (0.33, 13)**	**.01**					**0.79 (0.31, 1.3)**	**.004**
LOAD>Control					**0.90 (0.37, 1 4)**	**.003**					**0.98 (0.47, 1.5)**	**.0004**
EOAD>LOAD					0.18 (−0.69, 1.1)	.91					−0.19 (−0.69, 0.31)	.74
Inflammatory factor												
Model	0.14	6.1 (3, 98)		.0007			0.08	5.5 (2, 98)		.006		
Age			0.06	.01					–	–		
Group			0.02	.32					0.10	.006		
EOAD>Control					0.38 (−0.35, 1.1)	.56					−0.34 (−0.81, 0.14)	.34
LOAD>Control					0.30 (−0.21, 0.81)	.48					0.50 (0.01, 0.99)	.11
EOAD>LOAD					0.08 (−0.79, 0.94)	.98					**−0.84 (−1.35, −0.33)**	**.004**

NOTE. ANCOVA models adjusted for age with partial eta squared values reported for model parameters and Cohen's d for estimates of pairwise effect sizes. ANOVA models illustrate group effects per analyte without covariates with model eta squared and pairwise Cohen's d as effect size estimates. Independent variables that are significantly associated with the dependent variables are bolded. Abbreviations: EOAD, early-onset Alzheimer's disease; LOAD, late-onset Alzheimer's disease; ANOVA, analysis of variance; HSD, honestly significant difference; CI, confidence interval.

TABLE 5

Associations of factors with clinical and imaging outcomes

	CDRsob	MMSE	Memory	Executive	Spatial	Semantic	Processing speed	GMV
Model	F (2 87) = 2.6	F (2.83) =7.1 **	F (2, 73) = 5.2 **	F (2, 57) = 6.6 **	F (2.68) =5.9 **	F (2.75) =3.1	F (2.67) = 6.7 **	F (3. 65) = 3.0 *
TIV	–	–	–	–	–	–	–	0.30 *
Age	–0.20	0.38 **	0.31 **	0.38 **	0.18	0.14	0.36 **	–0.14
Degenerative factor	0.11	–0.05	–0.18	–0.24	**–0.33** **	**–0.24** *	–0.20	–0.16
Model	F (2 87) =10.1 **	F (2.83) =42.2 **	F (2, 73) = 28.8 **	F (2, 57) = 25.5 **	F (2. 68) = 5.5 **	F (2.75) =26.8 **	F (2.67) = 124 **	F (3. 65) = 10.8 **
TIV	–	–	–	–	–	–	–	0.33 **
Age	–0.22	0.37 **	0.30 **	0.33 **	0.19	0.16	0.32 **	–0.13 **
Trophic factor	**0.38** **	**–0.60** **	**–0.60** **	**–0.58** **	**–0.33** **	**–0.63** **	**–0.38** **	**–0.49** **
Model	F (2 87) = 4.4 *	F (2.83) =6.9 **	F (2, 73) = 3.9 *	F (2, 57) = 5.2 **	F (2. 68) = 1.4	F (2.75) = 1.2	F (2.67) = 5.8 **	F (3. 65) = 2.5
TIV	–	–	–	–	–	–	–	0.33 *
Age	–0.31 **	0.39 **	0.33 **	0.42	0.17	0.18	0.42 **	–0.12
Inflammatory factor	**0.24** *	–0.02	**–0.08** **	–0.16	0.05	–0.10	–0.16	–0.08

NOTE. The table illustrates model fit parameters and standardized β; the significance levels are indicated by stars as follows:

*
$P<.05$;

**
$P<.01$.

Independent variables that are significantly associated with the dependent variables are bolded.

Abbreviations: CDRsob, Clinical Dementia Rating, sum of boxes; MMSE, Mini–Mental Status Examination;GMV, total graymatter volume; TIV, total intracranial volume

TABLE 6

ANCOVA models comparing factors across groups classified by AD/WMH

	Model				Post hoc (Tukey HSD)	
	Adj. R²	F (df)	Partial η²	P value	Cohen's d (95% CI)	P value
Degenerative						
Model	0.06	3.2 (3, 98)		.03		
Age			0.002	.67		
Group			**0.09**	**.01**		
AD+WMH>Control					**0.72 (0.24, 1.2)**	**.01**
AD−WMH>Control					0.40 (−0.17, 0.97)	.34
AD+WMH>AD−WMH					0.32 (−0.26, 0.90)	.53
Trophic						
Model	0.15	6.6 (3, 98)		.0004		
Age			0.006	.46		
WMH Group			**0.17**	**.0001**		
AD+WMH>Control					**1.0 (0.51, 1.5)**	**.0002**
AD−WMH>Control					**0.86 (0.27, 1.4)**	**.01**
AD+WMH>AD−WMH					0.16 (−0.42, 0.74)	.85
Inflammatory						
Model	0.14	6.4 (3, 98)		.0005		
Age			**0.09**	**.002**		
WMH Group			0.03	.22		
AD+WMH>Control					0.42 (−0.05, 0.90)	.19
AD−WMH>Control					0.16 (−0.41, 0.72)	.64
AD+WMH>AD−WMH					0.26 (−0.31, 0.85)	.85

NOTE. ANCOVA models adjusted for age with partial η² values reported for model parameters and Cohen's d for estimates of pairwise effect sizes. Significant independent predictors are bolded.

Abbreviations: AD, Alzheimer's disease; WMH, white matter hyperintensity; CI, confidence interval; HSD, honestly significant difference; AD − WMH, Alzheimer's disease without white matter hyperintensity (Fazekas score 0–1); AD + WMH, Alzheimer's disease with white matter hyperintensity (Fazekas score 2–3).