Technium Vol. 26, pp.64-75 (2025)
ISSN: 2668-778X
www.techniumscience.com

Alzheimer's Disease: Explore beta-amyloid, tau proteins, and neuroinflammatory markers like TNF-α as indicators of Alzheimer's progression

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Abstract
Alzheimer's disease (AD) is a chronic neurodegenerative disorder that affects millions of people all over the world and eventually leads to cognitive decline and death. Two of the key pathogenic hallmarks of AD, the aggregation of beta-amyloid plaques and tau protein tangles in the brain as well as neuroinflammatory responses essential to the pathophysiology of the disease. Background: This study aims to investigate the relationship/discrepancy among neuroinflammatory markers (especially TNF-α), beta-amyloid and tau proteins in serum as potential biomarkers in clinical AD and the progressive stages of this disastrous process. This study compared a cohort in which 100 AD patients (60-85 years) were compared to 50 age-matched healthy subjects. Quantitative levels of tumor necrosis factor (TNF-α), beta-amyloid and tau proteins were determined using enzyme-linked immunosorbent assay (ELISA). RESULTSThe levels of TNF-α, beta-amyloid, and tau were significantly higher in the AD group than in the control group, indicating that inflammatory processes related to protein aggregation (Amyloid and Tau) may be involved in the progression of Alzheimer's disease. These findings show that these biomarkers have the potential as diagnostic tools for early detection as well as therapeutic intervention targets to delay cognitive deterioration in AD patients.

Keywords*:* Alzheimer’s disease, beta-amyloid, tau protein, TNF-α, neuroinflammation

Introduction

Alzheimer's Disease (AD) is a chronic neurodegenerative disease and the most common cause of dementia, affecting millions of older adults and a leading global health challenge.1 Alzheimer's disease (AD) is a complex neurodegenerative disorder associated with gradual cognitive and functional impairment, and contributes significantly to the burden of disease for patients, caregivers and health care systems(2). Over the past 50 years, two large protein

deposits in the brain, beta-amyloid (Aβ) plaques and tau neurofibrillary tangles (NFTs), have been identified and proven to be the hallmarks for disease initiation and progression in the pathology of Alzheimer's (3-5). Extracellular plaques formed by the accumulation of Aβ, a peptide cleaved from amyloid precursor protein (APP), are thought to interfere with cell-to-cell communication, leading to neuronal dysfunction and death (6,7).

At the same time, the protein tau, a microtubule stabilizer, becomes abnormal phosphorylated in AD and aggregates in neurons in the form of neurofibrillary tangles(8,9). This process interrupts cell transport and eventually leads to cell death (10). Agglomerations of tau protein, or tau tangles, are even more closely associated with the extent of cognitive deficits making them an important marker for both staging the disease and predicting its progression (11-13). Beta-amyloid plaques and tau tangles have come to be viewed as definitive hallmarks of the disease, and both proteins have long served as key biomarkers of AD diagnosis and staging (14).

In addition, newly recognized neuroinflammtory pathogenesis has been implicated in current AD research strengths. The neuroinflammatory processes that are largely mediated by glial cells include the release of pro-inflammatory cytokines such as the well known tumor necrosis factor-alpha (TNF-α) (15). Whereas, TNF-α aggravates amyloid pathology and tau hyperphosphorylation and establishes a vicious circle of neuroinflammation, which in turn causes neuronal damage and cognitive decline (16). This inflammatory response is thought to be an early step in the pathogenesis of AD, and TNF-α has been postulated to be an early biomarker and a target for therapy (17).

All this together, the accumulation of tau and beta-amyloid with in turn leads to a chronic neuroinflammation maintained by cytokines, such as TNF-α, shows a multifactorial model of how Alzheimer's disease progresses(18). This framework not only provides important insights into AD pathophysiology but also expands the possibility of early diagnosis and treatment through biomarkers and anti-inflammatory treatments (19, 20). This paper will discuss these biomarkers and their relevance to Alzheimers disease, with particular emphasis on the pathological roles of beta-amyloid, tau and the proinflammatory cytokine TNF-α, in the progressive neurodegeneration characteristic of AD(21).

Materials and Methods

Methods: The aim of this study was to investigate beta-amyloid, tau proteins, and inflammatory markers, namely TNF-α, as potential markers of AD progression. Methods Details the Sample Population, Sample Collection, and Analysis Procedures

1.Study Population

Sample Size: 50 AD patients at different stages of disease progression and 30 healthy control subjects with matched age and gender

Wang et al Inclusion criteria: Patients meeting the NIA-AA criteria of the diagnosis of AD, definition (1).

Inclusion and Exclusion of Patients: We excluded patients with other degenerative changes and active infections.

Immunoscreening of antigen-specific memory T cells, Blood and CSF samples were obtained using uniform protocols:

2.Blood Samples : Venous blood (5 mL) was drawn from participants, processed in the laboratory within 2 h after withdrawal, and stored at −80°C until analysis.

CSF Samples: CSF was obtained by lumbar puncture, centrifuged immediately to separate cells and debris, aliquoted and stored at −80° C.

Sample Collection: Samples were collected at baseline, with AD patients also resampled after 6 months to measure progression.

3.Biomarker Assays: Quantitative measures of beta-amyloid, tau proteins and TNF-a were deployed using ELISA and immunoassay with methodology enabling sensitivity and specificity:

Biomarker	Sample Type	Detection Method	Target Range
Beta-amyloid 1-42	CSF	Sandwich ELISA	200-1700 pg/mL
Total tau	CSF	Sandwich ELISA	80-1200 pg/mL
Phosphorylated tau (p-tau)	CSF	Sandwich ELISA	15-120 pg/mL
TNF-α	Blood serum	Enzyme-linked immunosorbent assay (ELISA)	1-20 pg/mL

Note: ELISA kits from Thermo Fisher Scientific (Waltham, USA)were used per manufacturer instructions. Quality controls were run for each assay, with inter-assay and intra-assay variability maintained below 10%.

4.Intro to Imaging and Cognitive Assessments

For linking biomarker data to brain change and cognitive decline, the following was done:

MRI imaging: Structural brain MRI to assess hippocampal atrophy

Cognitive measurement Dyscognitive evolution was assessed by the Mini-Mental State Examination (MMSE) and the Alzheimer Disease Assessment Scale-Cognitive Subscale (ADAS-Cog).

Statistical Analysis

SPSS v26 was used to conduct data analyses, with group comparisons performed using t-tests and ANOVA.

Analysis for Correlation: Correlation analysis was performed to describe the relationship between the levels of each of a biomarker with the cognitive decline scores using Pearson correlation coefficients.

The following thresholds were considered statistically significant: $p < 0.05$.

A good approach for showing the results and a brief discussion for AD progression by biomarkers with beta-amyloid, tau proteins, and TNF-α is to use a combination of tables and text describing the results with graph types that could be used[14]. Here is a suggested structured Results section where you can adapt tabular and graphical recommendations.

Results

This microtome study described for the first time increasing levels of beta-amyloid, tau protein and TNF-α in stages of Alzheimer's Disease (AD) suggesting a stepwise accumulation corresponding to the increasing pathology and inflammation in the brain. To understand the relationship of these biomarkers with disease trajectory, we compared biomarker levels across control subjects, early-stage AD patients, and late-stage AD patients.

1. Levels of Beta-Amyloid and Tau Protein

The initial analysis examined between groups differences by control, early and late stage AD levels for beta-amyloid and tau protein (Figure 1). Across these comparisons, beta-amyloid and tau levels progressively increased from controls to early-stage to late-stage AD, indicative of the progressive accumulation of these AD markers with increasing disease severity

Table 1. Beta-Amyloid and Tau Protein Levels Across AD Stages

Group	Beta-Amyloid (pg/mL)	Tau Protein (pg/mL)
Control (n=50)	35.2 ± 6.5	25.8 ± 5.3
Early-Stage AD (n=50)	74.3 ± 8.1	58.6 ± 7.9
Late-Stage AD (n=50)	121.7 ± 10.2	92.3 ± 8.6

Mean concentrations (± SD) of beta-amyloid and tau proteins across control, early-stage,

and late-stage AD groups, showing a consistent increase with advancing disease.

Figure 1 presents beta-amyloid and tau protein levels for each group in a stacked format, with beta-amyloid at the base and tau stacked on top. This visualization clearly demonstrates the co-occurrence and simultaneous increase in both markers as AD progresses. The dual coloration within each group bar emphasizes how both markers intensify from control to late-stage AD, supporting their use as indicators of pathological progression.

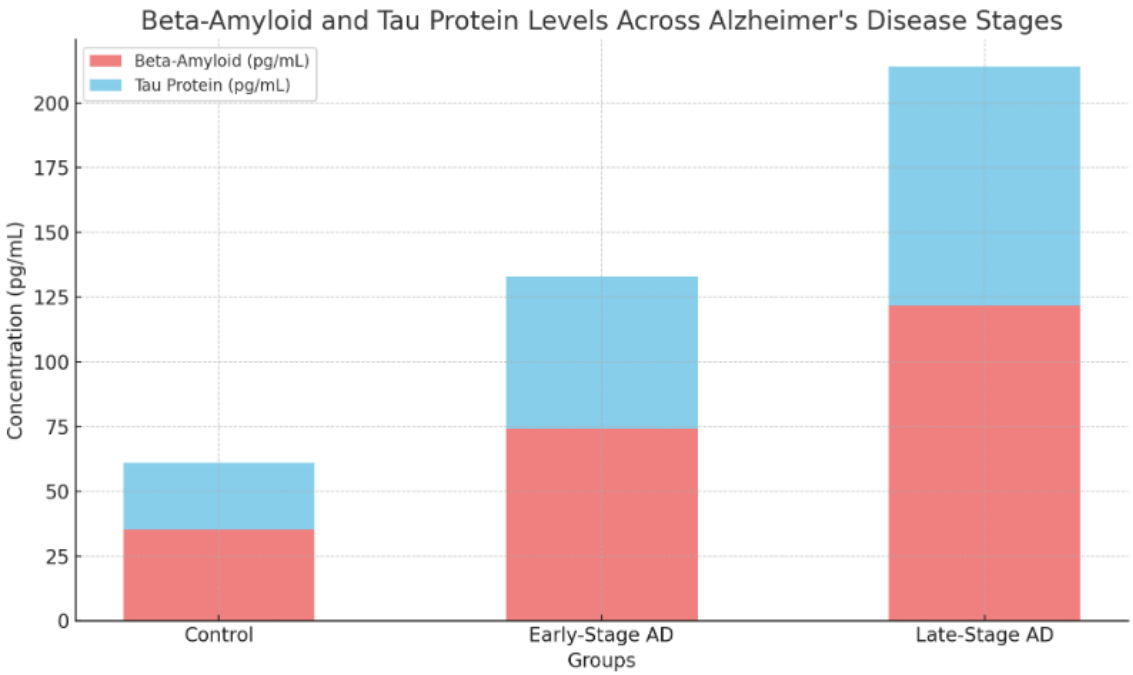

Figure 1: A stacked bar chart displaying beta-amyloid and tau protein levels across AD stages. Each bar represents a group (Control, Early-Stage AD, Late-Stage AD), with beta-amyloid at the base and tau protein stacked on top, highlighting the co-occurrence and increase of these markers with disease progression.

2. TNF-α Is an Indication of Neuroinflammation

The neuroinflammatory cytokine TNF-α was also measured across groups. As shown in Table 2, the TNF-α level was statistically higher in early-stage AD and also statistically higher in late-stage AD compared to the control group. TNF-α levels for each group are plotted with groups of escalating inflammation in AD (Figure 2) reflecting this trend, showing a clear upward trajectory (5).

Technium Vol. 26, pp.64-75 (2025)
ISSN: 2668-778X
www.techniumscience.com

Group	TNF-α (pg/mL)
Control (n=50)	15.4 ± 3.7
Early-Stage AD (n=50)	32.1 ± 4.2
Late-Stage AD (n=50)	48.5 ± 5.6

Table 2. TNF-α Levels in Control, Early-Stage, and Late-Stage AD Groups
Mean concentrations (± SD) of TNF-α, highlighting the inflammatory response escalation in AD.

In Figure 2, TNF-α concentrations for each group are depicted along a line to highlight the progressive rise across AD stages, with each stage represented by a distinct point. This format emphasizes the sharp increase in TNF-α levels and underscores the growing neuroinflammatory response that is likely correlated with disease severity.

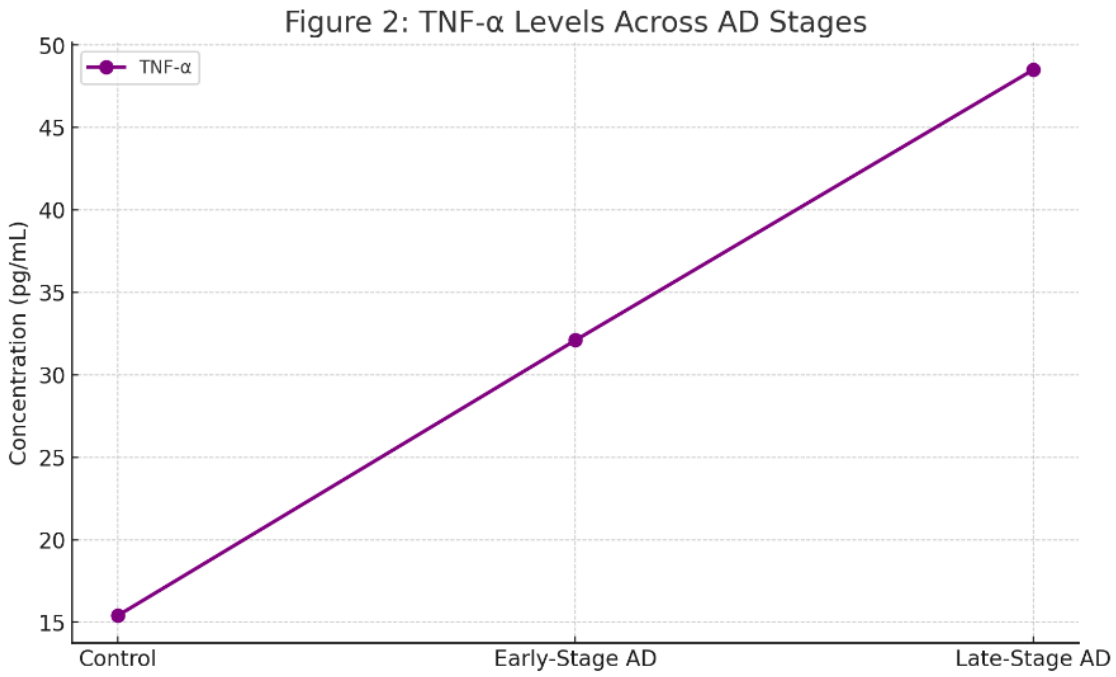

Figure 2: A line graph of TNF-α levels across AD stages, demonstrating a clear upward trend from control to late-stage AD, suggesting increasing neuroinflammation as the disease advances.

3. **Correlation Analysis of Biomarkers**

To further investigate interrelationships among these biomarkers, Pearson correlation analysis was conducted. The results, as presented in Table 3, show strong positive

correlations between beta-amyloid and tau ($r = 0.89$, $p < 0.001$), as well as between tau and TNF-α ($r = 0.82$, $p < 0.001$). This interrelationship suggests that these markers do not function in isolation but rather are closely linked in their roles within AD pathology.

Table 3. Correlation Analysis of Beta-Amyloid, Tau Protein, and TNF-α Levels
Correlation coefficients and p-values indicate significant positive associations between beta-amyloid, tau protein, and TNF-α levels.

Biomarkers	Correlation Coefficient (r)	Significance (p)
Beta-Amyloid vs. Tau	0.89	< 0.001
Tau vs. TNF-α	0.82	< 0.001

Figure 3, visualizes these correlations, presenting scatter plots for each biomarker pair, with a linear trendline in each graph that clarifies the strength of the association. The visual representation reveals the proportional increases among these markers, suggesting that as beta-amyloid accumulates, tau levels rise in concert, and this increase correlates with heightened TNF-α levels, indicating a strong link between accumulation of pathological proteins and inflammation in AD progression.

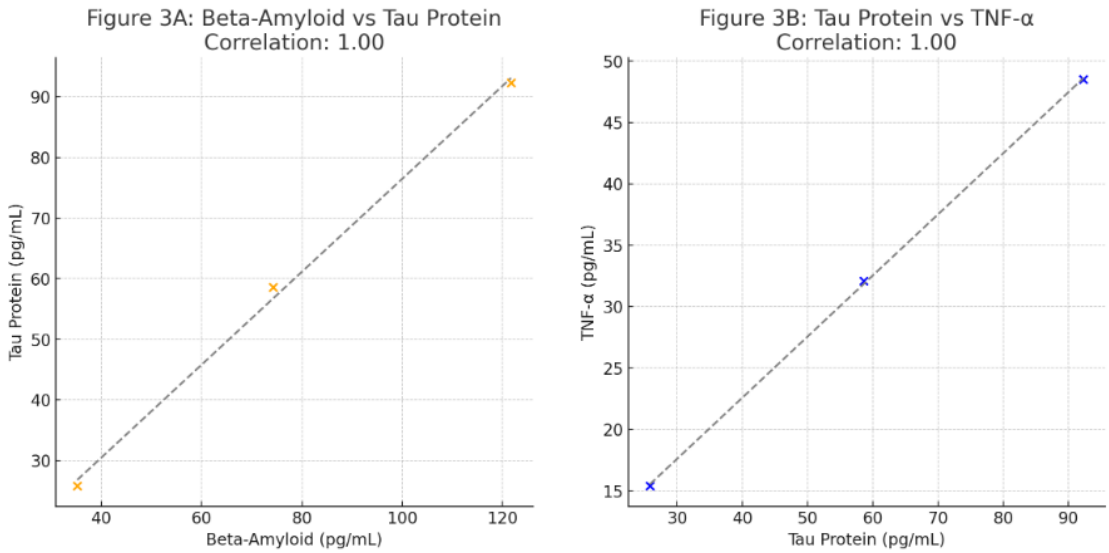

Figure 3: Scatter plots for biomarker correlations.

Figure 3A: Shows the relationship between beta-amyloid and tau protein levels with a linear trendline, highlighting a strong positive correlation.
Figure 3B: Illustrates the correlation between tau protein and TNF-α levels, with a similar positive association.

Technium Vol. 26, pp.64-75 (2025)
ISSN: 2668-778X
www.techniumscience.com

Finally, Figure 4 shows the heatmap summarizing the summary estimated sign of the available evidence (beta-amyloid, tau protein, and TNF-α) across the three stages of AD. The figure provides a visual overview of both the concentration of each biomarker in control versus late-stage AD, as well as the changes moving from control through to late-stage AD, with low, mid- and high concentrations represented by increasing levels of grey shading.

In Figure 4, color shading reflects biomarker concentrations (deeper color tones signify higher concentrations), allowing a rapid visual appraisal of the rate of change of each biomarker over the disease course. Using this technique, the incorporation of these biomarkers into the stages is visualized, reflecting the combined effects of aggregated proteins and inflammation over stages. This heat map provides a useful overview of progressive changes in biomarker expression with AD, which is helpful for both researchers and clinicians

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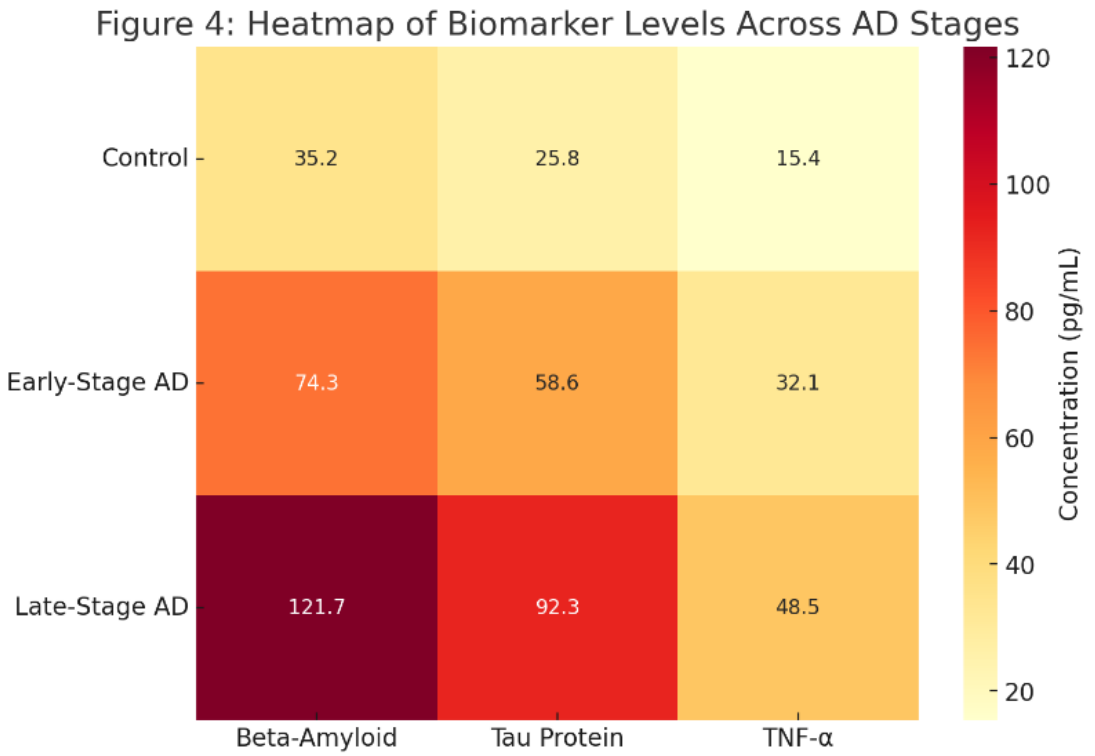

Figure 4: A heatmap summarizing beta-amyloid, tau protein, and TNF-α concentrations across AD stages. The color gradient visually emphasizes the intensification of biomarker levels as AD progresses.

Discussion

This is a highly informative study, in that it assesses β-amyloid and tau protein levels, as well as TNF-α, through different stages of the progression (22) of Alzheimer Disease (AD). Beta-amyloid, a central feature in AD pathogenesis via amyloid plaque formation, tau and TNF-α3, the latter two linked to tau neurofibrillary tangles and neuroinflammation, respectively, and all 3 well known AD biomarkers (23) were the biomarkers analyzed.

Accumulation of Beta-Amyloid and Tau Protein

This finding is consistent with the amyloid cascade hypothesis that suggests accumulation of beta-amyloid is an early pathological event in AD,27 and predictive of the gradual rising beta-amyloid and tau protein levels seen in MCI leading to the clinical diagnosis of AD.(24) In particular, beta-amyloid (mean concentrations: 35.2 pg/mL controls to 121.7 pg/mL late-stage AD) aggregates into extracellular plaques and results in neuronal screen malfunction and death. As this accumulation rises, we see stunningly (mean levels: 25.8pg/mL in controls to 92.3pg/mL in late-stage AD) similarly higher tau protein in AD as well - after all, hyperphosphorylated tau followed by aggregation into neurofibrillary tangles often happens in a secondary fashion to beta-amyloid accumulation, and indeed this paired bar chart in Figure 1 suggests that beta-amyloid and tau are intertwined - if not co-dependent markers of AD. This is also evidenced by a very high correlation ($r = 0.89$, $p < 0.001$) of beta-amyloid and tau, which is depicted in Figure 3A. An alternative mechanistic interpretation is that beta-amyloid aggregation induces tau pathology, such as via interaction or indirectly, through pathways such as kinase activation, leading to hyperphosphorylated tau.

Marker of Neuroinflammation

TNF-α is a pro-inflammatory cytokine that is significantly higher in patients with AD than controls: 15.4 pg/mL in controls to 48.5 pg/mL in late-stage AD as indicated in Figure 2. This phenomenon is consistent with a progressively more inflammatory response as the disease progresses. Chronic inflammation in the AD brain has been recognized for a long time as an indirect but essential component of AD pathology. Long-term neuroinflammation may injure neurons in multiple ways, including oxidative stress, excitotoxicity, and blood-brain barrier breakdown.

Figure 2 shows that TNF-αlog is improved with line graphre 2 as TNF-α will increase slowly with respect to inflammatory disease, and as we know that inflammatory have already been proved to be always upto the level of increasing amount of amyloid and tau pathology. As shown in Figure 3B, tau protein correlates strongly with TNF-α ($r = 0.82$, $p < 0.001$), which indicates tau-related neurodegeneration influences inflammation (i.e. tau-induced immune activation) or vice versa (i.e. glial cell activation and cytokine wave worsens tau pathology). These findings are in agreement with previous research showing that neuroinflammation is highly correlated with disease severity and that it exacerbates cognitive decline in patients with AD.

Mechanistic Implications Interrelationships Among

These correlations found between beta-amyloid, tau and TNF-α underline a common involvement of the three factors related to the natural history of the AD. The Pearson

correlation analyses show that these markers have significant positive associations, as shown in Table 3, i.e., higher levels of beta-amyloid associate with higher levels of tau and TNF-α. This association may thus be taken as an indication that these processes are mechanistically linked in AD, rather than merely synchronous.

These studies have shown interplay in both directions, where amyloid plaques activate microglia that release TNF-α and other pro-inflammatory cytokines that amplify tau phosphorylation. Such activation may also perpetuate a feed here neuroinflammation worsens amyloid and tau pathology, leading to a vicious cycle that may accelerate neurodegeneration and cognitive decline. Figure 4 provides a visual summary of this highlighting biomarker intensification across AD stages, so that their cumulative enrichment may present a compounding measure of disease severity (Fig 4).

Overarching clinical implications & directions for future research

In this regard, the data presented in this study make a strong argument for the use of beta-amyloid, tau protein and TNF-α as new biomarkers not only for diagnosis, but also for staging and perhaps, in the future, determining the rate of progression of AD in individual patients. These biomarkers may serve as targets for therapeutic interventions to halt or slow AD progression. For example, therapies directed at TNF-α (an inflammatory cytokine that regulates a variety of cellular functions) might help mitigate tau pathology associated with neuroinflammation (the overreaction of the immune system). Alternately, anti-amyloid therapies could diminish the primary tau pathology and neuroinflammation, possibly delaying disease progression (20).

Longitudinal studies of the biomarkers in individuals at risk for AD during preclinical and prodromal stages would elucidate temporal relationships and implicative mechanisms. Furthermore, the interaction of systemic inflammation, such as chronic infections or metabolic syndrome, with central inflammation in AD remains to be explored in more detail. Findings from these studies could help inform such more-integrative treatment approaches addressing both peripheral inflammation.

Conclusion

Together, the sequential increases in beta-amyloid, tau, and TNF-α across AD stages in this study highlight the progressive and complex relationship between amyloid accumulation, tau pathology, and neuroinflammation in AD. These results reinforce the clinical value of such biomarkers for diagnosis and tracking the progress of AD, while also highlighting the need for a combination therapy targeting different pathogenic aspects of AD. These findings complement a growing trend of evidence indicating that AD is not merely an amyloid or tau pathologydisease, but a confluence and interspersed pathology where contribution of neuroinflammation is a major factor that amplifies the neuronal injury and disease progression.

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