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Association between Peripheral Immune-inflammatory Biomarkers and Response to Cognitive Rehabilitation in Early Alzheimer Disease

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ABSTRACT

This prospective single-arm study examined associations between peripheral immune-inflammatory biomarkers and changes observed during cognitive rehabilitation in patients with early Alzheimer disease.

Seventy-two patients completed a 12-week structured cognitive rehabilitation program delivered three times weekly. Baseline plasma/serum glial fibrillary acidic protein (GFAP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) were measured. Cognitive and functional outcomes were assessed before and after the program. Correlation and multivariable linear regression analyses examined associations between baseline biomarkers and pre–post changes.

After 12 weeks, scores on global cognition, memory, digit span, and semantic fluency increased, whereas Trail Making Test completion times and Activities of Daily Living dependency scores decreased. Among 60 participants with repeat biomarker measurements, GFAP, IL-6, TNF-α, and CRP levels were lower at follow-up. Baseline GFAP, IL-6, TNF-α, and CRP were inversely correlated with change in Montreal Cognitive Assessment score; GFAP showed the strongest correlation ($r=-0.48$). After adjustment for age, education, and baseline cognition, baseline logGFAP, logIL-6, and TNF-α remained associated with smaller Montreal Cognitive Assessment score changes.

In this uncontrolled cohort, cognitive rehabilitation was associated with short-term cognitive, functional, and biomarker changes. Higher baseline inflammatory biomarker levels were associated with smaller pre–post changes, with GFAP showing the strongest association. These exploratory findings do not establish efficacy or causality and require confirmation in randomized controlled studies.

Keywords: Alzheimer disease; Biomarkers; Cognitive training; Glial fibrillary acidic protein; Inflammation

INTRODUCTION

Alzheimer disease (AD) is the most common neurodegenerative cause of dementia and remains a major source of cognitive disability, functional

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dependence, caregiver burden, and healthcare expenditure worldwide.[1] With progressive population aging and the emergence of disease-modifying strategies, the field has increasingly shifted from passive post-diagnostic management toward early identification, biologically informed staging, risk stratification, and timely intervention.[2,3] This conceptual transition reflects the view that AD should be understood not only as a dementia syndrome, but also as a biological process that may begin years before overt clinical symptoms.[1,3]

Within the conventional framework, amyloid-β (Aβ) deposition and tau pathology have long dominated AD research. However, mounting evidence indicates that immune-inflammatory processes are not simply late epiphenomena of neurodegeneration, but active participants throughout disease initiation and progression.[4,5] Aberrant activation of microglia and astrocytes can alter cognition and disease trajectory through cytokine release, dysregulated synaptic pruning, blood-brain barrier disruption, and loss of network homeostasis.[6,7] Accordingly, integrating inflammation into the AD continuum has become a major direction in contemporary mechanistic and translational research.[5]

Compared with cerebrospinal fluid (CSF) testing and positron emission tomography (PET), blood-based biomarkers are less invasive, less costly, and more suitable for repeated longitudinal assessment.[3,6,7] Core plasma biomarkers such as p-tau217, p-tau181, the Aβ42/Aβ40 ratio, and neurofilament light chain already show strong utility for reflecting AD pathology, whereas inflammatory biomarkers—including glial fibrillary acidic protein (GFAP), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP)—may further complement disease phenotyping from an immune-inflammatory perspective.[7–11] Among these, GFAP has shown particular promise for early-stage stratification, while peripheral cytokines and acute-phase proteins may capture systemic inflammatory burden relevant to cognitive decline and disease heterogeneity.[8–11]

At the therapeutic level, cognitive rehabilitation, cognitive training, and cognitive stimulation remain important components of early AD management.[12,13] Unlike interventions focused solely on improving test scores, cognitive rehabilitation emphasizes individualized goals, strategy training, and transfer to real-world functioning.[12] Previous syntheses suggest that such interventions can improve global cognition, goal-directed daily activities, and selected functional outcomes in dementia or mild cognitive impairment, with some evidence of durability over longer follow-up.[13,14] Nevertheless, marked inter-individual variability is consistently observed in clinical practice, as similar training content and duration do not necessarily yield comparable degrees of benefit.[14]

The efficacy of cognitive rehabilitation depends on residual neural plasticity, learning consolidation, and the transfer of strategies across settings. Theoretically, patients entering rehabilitation with greater inflammatory burden or more pronounced glial reactivity may already have compromised synaptic plasticity and network reorganization, thereby limiting their capacity to benefit from training.[4,10,15] Baseline inflammatory status may therefore provide a biologically meaningful explanation for heterogeneity in rehabilitation response.[16]

Against this background, the present prospective self-controlled study focused on patients with early AD, measured peripheral immune-inflammatory biomarkers, and examined cognitive and functional changes before and after a 12-week structured cognitive rehabilitation program. We hypothesized that higher baseline inflammatory burden would be associated with smaller rehabilitation gains, and that GFAP might show a relatively stronger association than the other measured markers.

MATERIALS AND METHODS

Study Design and Participants

This was a prospective self-controlled before–after study conducted at The Third People's Hospital of Chengdu, Chengdu, Sichuan Province, China. Participants were recruited from the Department of Neurology, memory clinic, Department of Geriatrics, and related nursing follow-up services at the same institution between January 2022 and January 2024. The diagnosis of early Alzheimer disease was established by at least two attending physicians with expertise in cognitive disorders, based on clinical history, neuropsychological assessment, brain magnetic resonance imaging, and available biomarker data. Early AD was defined as the mild cognitive impairment stage or mild dementia stage within the AD continuum. All included participants completed baseline inflammatory biomarker testing and pre/post cognitive assessment; 60 participants additionally completed post-intervention biomarker reassessment. Written informed consent was obtained from each participant or legal guardian.

Inclusion and Exclusion Criteria

Inclusion criteria were as follows: (1) age 55–85 years; (2) fulfillment of diagnostic criteria for early AD, including amnestic mild cognitive impairment with AD pathological support or mild AD dementia; (3) Clinical Dementia Rating (CDR) score of 0.5–1.0; (4) Mini-Mental State Examination (MMSE) score of 18–26; (5) stable anti-dementia and cognition-relevant medication regimens during the preceding 4 weeks; (6) sufficient visual, auditory, and language abilities to complete rehabilitation and assessment; and (7) ability of both the participant and primary caregiver to comply with follow-up and training records. Exclusion criteria included: (1) other dementia subtypes or central nervous system diseases clearly affecting cognition; (2) stroke, traumatic brain injury, or status epilepticus within 6 months; (3) severe psychiatric illness or overt delirium; (4) acute infection, active autoimmune disease, malignancy, or severe hepatic/renal dysfunction that could markedly influence inflammatory status; (5) glucocorticoid, immunosuppressant, or biologic therapy within 1 month; (6) severe sensory or speech impairment; and (7) anticipated completion of fewer than 80% of training sessions.

Biomarker Measurement

Peripheral venous blood (10 mL) was collected in the fasting state on the morning before intervention. Five milliliters were placed in EDTA tubes for plasma GFAP measurement, and 5 mL were placed in serum separator tubes for IL-6, TNF-α, and CRP testing. When feasible, blood collection was repeated after completion of the intervention to evaluate biomarker dynamics. Samples were centrifuged at 3000 rpm for 10 min within 2 h after collection, and supernatants were stored at −80 °C without repeated freeze–thaw cycles.

Plasma GFAP was measured using a Simoa HD-X Automated Immunoassay Analyzer (Quanterix Corporation, Billerica, MA, USA) with the Simoa GFAP Discovery Kit (Item No. 102336). The manufacturer-reported within-run and between-run coefficients of variation ranged from 6.3% to 10.9% and from 5.7% to 13.6%, respectively.

Serum IL-6 and TNF-α concentrations were measured using sandwich enzyme-linked immunosorbent assay kits (Human IL-6 ELISA Kit, catalog No. E-EL-H6156; Human TNF-α ELISA Kit, catalog No. E-EL-H0109; Elabscience Biotechnology Inc., Wuhan, China) according to the manufacturer's instructions. The manufacturer-reported intra-assay and inter-assay coefficients of variation were 5.06%–5.96% and 6.06%–8.49%, respectively, for IL-6, and 4.53%–6.10% and 6.03%–8.18%, respectively, for TNF-α. Optical density was measured at 450±2 nm using a microplate reader.

Serum CRP was measured using a particle-enhanced immunoturbidimetric assay on a cobas c 311 automated clinical chemistry analyzers with the Tina-quant C-Reactive Protein IV reagent (catalog No. 07876033190; Roche Diagnostics GmbH, Mannheim, Germany). The assay was performed according to the manufacturer's instructions.

Baseline and post-intervention samples from the same participant were analyzed using the same assay method whenever paired samples were available. Samples were tested in duplicate, and the mean value was used for statistical analysis.

Cognitive Rehabilitation Protocol

All participants underwent a 12-week structured cognitive rehabilitation program, three sessions per week, 45–60 min per session, delivered by a multidisciplinary team including rehabilitation therapists, neuropsychological therapists, and trained nursing staff. The intervention was primarily individualized and supplemented, when necessary, by small-group sessions involving two to four participants. Training domains included: (1) orientation; (2) attention; (3) memory; (4) executive function; (5) language and visuospatial ability; and (6) ecologically valid daily-life tasks such as medication reminders, shopping lists, route recall, telephone use, and object management. Task difficulty was adjusted dynamically according to prior performance. Home-based practice was assigned concurrently and completed with caregiver support. Background pharmacological treatment and management of comorbidities remained relatively stable throughout the intervention period.

Outcome Measures

Baseline data included sex, age, years of education, disease duration, body mass index (BMI), smoking and drinking history, hypertension, diabetes mellitus, coronary heart disease, and long-term medication use. Inflammatory biomarkers included GFAP, IL-6, TNF-α, and CRP, with baseline levels serving as the main exposure variables. Cognitive and functional outcomes were assessed at baseline and after 12 weeks using the MMSE and Montreal Cognitive Assessment (MoCA)

for global cognition; the Auditory Verbal Learning Test (AVLT) immediate and delayed recall for memory; the Digit Span Test (DST) and Trail Making Test parts A and B (TMT-A and TMT-B) for attention/executive function; semantic fluency for language; and the 14-item Activities of Daily Living (ADL) scale (Chinese version of the 14-item Lawton‐Brody Activities of Daily Living scale), assessing physical and instrumental tasks, where higher scores indicate greater functional impairment. The primary outcome was change in MoCA score, calculated as post-intervention score minus baseline score. Secondary outcomes included changes in MMSE, AVLT, DST, TMT, and ADL measures.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM, Armonk, NY, USA) and R version 4.1 were used for statistical analysis. Normally distributed continuous variables are presented as mean±SD and were compared using paired or independent-samples t tests as appropriate. Non-normally distributed variables are presented as median (P25, P75) and were compared using the Wilcoxon signed-rank test or Mann–Whitney U test. Categorical variables are presented as n (%) and were compared using the χ^2 test or Fisher's exact test. Because TMT-A and TMT-B values were not normally distributed, they were presented as median (P25, P75) and compared using the Wilcoxon signed-rank test for paired pre- and post-intervention comparisons. Pearson or Spearman correlation analyses were used to assess associations between inflammatory biomarkers and rehabilitation gain. Multivariable linear regression models were constructed with baseline inflammatory biomarkers as independent variables and changes in MoCA, MMSE, and ADL as dependent variables. The final models adjusted for age, years of education, and baseline cognitive status. Skewed variables were log-transformed before model entry. For variables in which lower scores indicate better performance or less impairment (e.g., ADL, TMT-A, and TMT-B), improvement was calculated as baseline minus post-intervention value, so that higher positive values indicate greater rehabilitation gain. All statistical tests were two-sided, and $p<0.05$ was considered statistically significant.

RESULTS

Participant Flow and Baseline Characteristics

A total of 78 patients with early AD were initially screened. Six were excluded from the final analysis because of insufficient adherence, failure to complete the endpoint assessment, or withdrawal during the intervention, leaving 72 participants for the final statistical analysis and an overall completion rate of 92.31%. All included participants completed baseline inflammatory biomarker testing and pre/post cognitive assessment, and 60 also completed post-intervention biomarker reassessment. The low attrition rate suggests that participation in the rehabilitation program was generally manageable in this cohort.

The mean age of the cohort was 69.8±6.4 years, 61.1% were women, and the mean years of education were 9.6±3.8. Baseline MMSE and MoCA scores were 22.6±2.5 and 18.7±3.0, respectively. Disease duration was relatively short, and the overall comorbidity profile was broadly consistent with that of a typical older memory-clinic population. GFAP, IL-6, and CRP showed skewed distributions, indicating that logarithmic transformation or non-parametric methods would be appropriate for subsequent analyses. Overall, the sample mainly consisted of patients at the mild cognitive impairment or mild dementia stage, providing a suitable clinical basis for rehabilitation and the evaluation of between-patient variability in training response. Detailed baseline characteristics are shown in Table 1.

Changes in Cognitive and Functional Outcomes after Intervention

After 12 weeks of intervention, participants showed improvement across global cognition, memory, attention/executive function, and daily functioning. MMSE increased from 22.6±2.5 to 24.1±2.6, and MoCA increased from 18.7±3.0 to 21.0±3.2. AVLT immediate and delayed recall, DST forward and backward span, and semantic fluency all increased, whereas TMT-A time, TMT-B time, and ADL score all decreased. All comparisons reached statistical significance (all $p<0.001$).

Importantly, the intervention effect was not confined to a single global cognition measure. Instead, gains were observed across memory retrieval, working memory, information processing speed, executive control, and functional ability, suggesting that structured cognitive rehabilitation may produce meaningful cross-domain transfer rather than narrow test-specific improvement (Table 2).

Table 1. Baseline characteristics of the study population (n=72).

Variable	Value	Variable	Value
Age, years	69.8±6.4	MoCA score	18.7±3.0
Female, n (%)	44 (61.1)	AVLT immediate recall	16.8±4.2
Education, years	9.6±3.8	AVLT delayed recall	3.5±1.8
Disease duration, years	2.1±0.9	DST forward span	5.1±1.0
BMI, kg/m^2	23.9±3.0	DST backward span	3.2±0.9
CDR = 0.5, n (%)	41 (56.9)	TMT-A, s	88.6±25.4
CDR = 1.0, n (%)	31 (43.1)	TMT-B, s	201.4±61.3
Hypertension, n (%)	35 (48.6)	Semantic fluency, items/min	12.1±3.6
Diabetes mellitus, n (%)	17 (23.6)	ADL score	23.6±5.1
Coronary heart disease, n (%)	11 (15.3)	GFAP, pg/mL	217.5 (176.8, 261.4)
Cholinesterase inhibitor use, n (%)	46 (63.9)	IL-6, pg/mL	4.8 (3.7, 6.2)
Memantine use, n (%)	19 (26.4)	TNF-α, pg/mL	13.5±3.4
MMSE score	22.6±2.5	CRP, mg/L	3.2 (1.8, 4.7)

ADL: Activities of Daily Living; AVLT: Auditory Verbal Learning Test; BMI: body mass index; CDR: Clinical Dementia Rating; CRP: C-reactive protein; DST: Digit Span Test; GFAP: glial fibrillary acidic protein; IL-6: interleukin-6; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; TMT: Trail Making Test; TNF-α: tumor necrosis factor-α.

Table 2. Changes in cognitive and functional outcomes before and after intervention (n=72).

Variable	Pre-intervention	Post-intervention	Statistic	p
MMSE score	22.6±2.5	24.1±2.6	t=7.84	<0.001
MoCA score	18.7±3.0	21.0±3.2	t=9.21	<0.001
AVLT immediate recall	16.8±4.2	20.1±4.5	t=8.11	<0.001
AVLT delayed recall	3.5±1.8	4.8±1.9	t=6.34	<0.001
DST forward span	5.1±1.0	5.7±1.1	t=5.19	<0.001
DST backward span	3.2±0.9	3.9±1.0	t=6.01	<0.001
TMT-A, s	86.0 (71.0, 104.0)	75.0 (63.0, 90.0)	Z=−5.72	<0.001
TMT-B, s	198.0 (160.0, 239.0)	176.0 (145.0, 211.0)	Z=−5.10	<0.001
Semantic fluency	12.1±3.6	13.9±3.8	t=5.42	<0.001
ADL score	23.6±5.1	20.8±4.8	t=6.75	<0.001

Note: Data are presented as mean±SD or median (P25, P75), as appropriate. Paired t tests were used for normally distributed variables, and Wilcoxon signed-rank tests were used for non-normally distributed variables. For TMT-A and TMT-B, the statistic is reported as Z.

ADL: Activities of Daily Living; AVLT: Auditory Verbal Learning Test; DST: Digit Span Test; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; TMT: Trail Making Test.

Dynamic Changes in Peripheral Immune-inflammatory Biomarkers

Among the 60 participants from the final cohort of 72 who completed post-intervention biomarker reassessment, post-intervention levels of GFAP, IL-6, TNF-α, and CRP were lower than their respective baseline values (Table 3). Median GFAP decreased from 214.2 to 205.9 pg/mL, IL-6 from 4.7 to 4.2 pg/mL, mean TNF-α from 13.3±3.3 to 12.7±3.1 pg/mL, and median CRP from 3.1 to 2.8 mg/L. All differences were statistically significant.

These findings suggest that reduced peripheral inflammatory burden may accompany cognitive and functional improvement during rehabilitation. Nevertheless, the causal direction of this association cannot be inferred from the present design and would require confirmation in controlled longitudinal studies.

Stratified Analysis According to Baseline GFAP Level

Using the baseline median GFAP value (217.5 pg/mL), the 72 participants were divided into low-GFAP and high-GFAP groups. The two groups did not differ significantly in age, years of education, baseline MMSE, or baseline MoCA, suggesting reasonable comparability at study entry. When rehabilitation gains were compared, the low-GFAP group showed significantly greater improvement in MMSE, MoCA, and ADL than the high-GFAP group: 2.0±1.2 versus 0.9±1.0 for MMSE change, 3.1±1.5 versus 1.5±1.3 for MoCA change, and 3.7±2.1 versus 1.8±1.9 for ADL change (all $p<0.001$).

This exploratory subgroup analysis is consistent with the possibility that lower baseline glial reactivity is associated with greater cognitive and functional gain after rehabilitation. (Table 4).

Correlation between Baseline Biomarkers and Rehabilitation Gain

Correlation analyses showed that baseline GFAP, IL-6, TNF-α, and CRP were all negatively associated with change in MoCA score, with GFAP showing the strongest correlation ($r=-0.48$, $p<0.001$). Baseline GFAP, IL-6, and TNF-α were also negatively correlated with MMSE and ADL improvement, whereas the associations of CRP with MMSE and ADL change did not reach statistical significance.

Taken together, these findings indicate that the greater the inflammatory burden at baseline, the smaller the improvement in global cognition and daily function after rehabilitation. The pattern was most consistent for GFAP, suggesting that it may be a promising candidate marker for between-patient variability in rehabilitation response (Table 5).

Multivariable Regression Analysis for MoCA Change

In the multivariable linear regression model, after age, years of education, baseline MoCA, and inflammatory biomarkers were entered simultaneously, baseline logGFAP, logIL-6, and TNF-α remained independently associated with smaller MoCA improvement. Among them, logGFAP showed the largest absolute standardized β ($\beta=-0.31$, $p<0.001$), suggesting the most robust association with rehabilitation gain in the current dataset. Years of education emerged as an independent positive predictor, whereas baseline MoCA was a negative predictor, suggesting that greater educational reserve and lower initial global cognition may correspond to greater potential for improvement. LogCRP did not remain statistically significant in the adjusted model.

The adjusted R^2 of the model was 0.46, indicating that inflammatory biomarkers together with clinical covariates accounted for a substantial proportion of the variance in rehabilitation response (Table 6).

Table 3. Changes in peripheral immune-inflammatory biomarkers before and after intervention (n=60).

Variable	Pre-intervention	Post-intervention	Statistic	*p*
GFAP, pg/mL	214.2 (173.5, 257.1)	205.9 (166.4, 246.8)	Z=−2.50	0.012
IL-6, pg/mL	4.7 (3.6, 6.0)	4.2 (3.2, 5.3)	Z=−2.73	0.006
TNF-α, pg/mL	13.3±3.3	12.7±3.1	t=2.43	0.018
CRP, mg/L	3.1 (1.8, 4.5)	2.8 (1.6, 4.0)	Z=−2.31	0.021

CRP: C-reactive protein; GFAP: glial fibrillary acidic protein; IL-6: interleukin-6; TNF-α: tumor necrosis factor-α.

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Table 4. Rehabilitation outcomes stratified by baseline GFAP level.

Variable	Low GFAP (n=36)	High GFAP (n=36)	Statistic	*p*
Age, years	69.1±6.2	70.5±6.6	t=0.95	0.347
Education, years	9.9±3.7	9.3±3.9	t=0.68	0.500
Baseline MMSE	22.8±2.4	22.4±2.6	t=0.66	0.513
Baseline MoCA	18.9±3.1	18.5±2.9	t=0.51	0.612
MMSE change	2.0±1.2	0.9±1.0	t=4.26	<0.001
MoCA change	3.1±1.5	1.5±1.3	t=4.92	<0.001
ADL change	3.7±2.1	1.8±1.9	t=4.05	<0.001

ADL: Activities of Daily Living; GFAP: glial fibrillary acidic protein; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment.

Table 5. Correlations between baseline biomarkers and treatment response (n=72).

Variable	MoCA change, r (*p*)	MMSE change, r (*p*)	ADL change, r (*p*)
GFAP	−0.48 (<0.001)	−0.42 (<0.001)	−0.39 (0.001)
IL-6	−0.36 (0.002)	−0.31 (0.008)	−0.29 (0.013)
TNF-α	−0.33 (0.005)	−0.28 (0.017)	−0.24 (0.041)
CRP	−0.27 (0.022)	−0.21 (0.076)	−0.18 (0.127)

ADL: Activities of Daily Living; CRP: C-reactive protein; GFAP: glial fibrillary acidic protein; IL-6: interleukin-6; MMSE: Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; TNF-α: tumor necrosis factor-α.

Table 6. Multivariable linear regression analysis for change in MoCA score.

Variable	B	SE	Std β	95% CI	*p*
Constant	9.84	2.31	—	5.23 to 14.45	<0.001
logGFAP	−2.14	0.58	−0.31	−3.30 to −0.98	<0.001
logIL-6	−1.28	0.51	−0.24	−2.30 to −0.26	0.015
TNF-α	−0.08	0.03	−0.19	−0.15 to −0.01	0.023
logCRP	−0.51	0.37	−0.12	−1.25 to 0.22	0.167
Age	−0.05	0.02	−0.17	−0.10 to −0.01	0.041
Education, years	0.12	0.05	0.21	0.02 to 0.22	0.018
Baseline MoCA	−0.19	0.06	−0.29	−0.31 to −0.07	0.002

CI: confidence interval; CRP: C-reactive protein; GFAP: glial fibrillary acidic protein; IL-6: interleukin-6; MoCA: Montreal Cognitive Assessment; SE: standard error; TNF-α: tumor necrosis factor-α.

DISCUSSION

The present study yielded three main findings. First, after 12 weeks of structured cognitive rehabilitation, patients with early AD showed improvements in global cognition, memory, attention/executive function, and activities of daily living. Second, among those who underwent biomarker reassessment, post-intervention GFAP, IL-6, TNF-α, and CRP levels were modestly lower than baseline. Third, higher baseline GFAP, IL-6, and TNF-α levels were associated with smaller improvements in MMSE, MoCA, and ADL, with GFAP showing the most consistent signal across correlation and regression analyses in the current dataset. These findings suggest that peripheral immune-inflammatory status may be related to heterogeneity in cognitive rehabilitation response. However, because this was a self-controlled before–after study without a randomized

control group, these results should be interpreted as associative and exploratory rather than causal.

Regarding overall intervention efficacy, our findings are broadly consistent with the current evidence base for non-pharmacological interventions in dementia and mild cognitive impairment. Prior systematic reviews and meta-analyses have shown that cognitive rehabilitation and related cognitive interventions can improve global cognition, targeted activity performance, and selected functional outcomes. In the present study, gains were observed across memory, attention/executive domains, and ADL rather than being restricted to a single rating scale, which is more closely aligned with the functional orientation of cognitive rehabilitation.

The improvement observed after intervention should be interpreted with caution because the study did not include a randomized control group, active comparator, or usual-care control arm. Therefore, the observed changes may have been partly influenced by practice effects, spontaneous fluctuation, placebo-related effects, caregiver engagement, or changes in daily routines during follow-up. Although the multi-domain pattern of improvement and the concurrent ADL change support the clinical relevance of the findings, the present design cannot isolate the specific effect of cognitive rehabilitation from these potential influences.

The clinical significance of the observed cognitive changes also requires careful interpretation. In the present cohort, the mean MoCA increase was 2.3 points and the mean MMSE increase was 1.5 points after 12 weeks. These changes suggest measurable improvement at the group level, and the accompanying reduction in ADL scores provides additional functional context. However, minimal clinically important differences for MMSE and MoCA are not uniformly defined across early AD, mild cognitive impairment, and rehabilitation settings. Therefore, the present results should be interpreted as statistically significant and potentially clinically relevant, but not as definitive evidence of clinically meaningful improvement in every individual patient.

One notable finding of this study was the association between higher baseline inflammatory burden and smaller rehabilitation gains. From a mechanistic perspective, AD is not driven solely by a linear cascade of Aβ and tau abnormalities. Neuroinflammation may emerge early in the disease course and affect cognition through aberrant glial activation, disruption of synaptic homeostasis, blood-brain barrier dysfunction, and reduced efficiency of large-scale neural networks. Because successful cognitive rehabilitation depends on the capacity of residual neural systems to reorganize and compensate, inflammatory status may serve as an indirect window into the degree of impairment in 'plasticity reserve.' When inflammatory burden is higher at baseline, the ability to achieve network-level reorganization and functional compensation through training may already be constrained.

GFAP showed the most consistent association with rehabilitation gain in the current dataset, but this finding should be interpreted cautiously. GFAP is commonly regarded as a marker of astrocytic activation and has also been increasingly discussed as a marker related to AD pathology and neurodegenerative disease burden.[17] Therefore, elevated GFAP should not be interpreted simply as a modifiable inflammatory signal. It may reflect a combination of reactive astrocytosis, disease burden, and altered brain microenvironment. Astrocytes contribute to metabolic support, ion homeostasis, neurotransmitter recycling, synaptic plasticity, blood-brain barrier maintenance, and coordination of neuronal activity. Within this framework, higher baseline GFAP may indicate a less favorable biological background for cognitive compensation and training-related improvement. However, the present study cannot determine whether GFAP directly affects rehabilitation response or merely reflects underlying disease severity.

IL-6 and TNF-α were also associated with smaller rehabilitation gains, but these findings should not be interpreted as direct evidence of central neuroinflammation. Peripheral cytokines are nonspecific systemic markers and may be influenced by age, metabolic status, vascular comorbidity, psychological stress, sleep disturbance, subclinical infection, and medication use. They may be related to brain function through blood-brain barrier regulation, neurovascular-unit signaling, or systemic inflammatory load, but the present study did not include cerebrospinal fluid biomarkers, PET imaging, or other central measures to confirm central nervous system inflammatory activity. Therefore, IL-6 and TNF-α should be interpreted as peripheral immune-inflammatory correlates of rehabilitation response rather than direct proxies for central neuroinflammation.

CRP showed a negative association with MoCA improvement in univariable analysis but did not retain independent significance in multivariable regression. This pattern is clinically reasonable because CRP is a

broad and nonspecific marker of systemic inflammation. It may be affected by infection, obesity, metabolic syndrome, cardiovascular disease, sleep disturbance, and age-related low-grade inflammation. Therefore, CRP may be more appropriately interpreted as a general inflammatory-load marker than as a disease-specific predictor of cognitive rehabilitation response.

The modest post-intervention reduction in inflammatory biomarkers should be interpreted cautiously. Cognitive rehabilitation may indirectly alleviate stress-related inflammatory activation by increasing behavioral engagement, improving perceived control, supporting social interaction, and stabilizing daily routines. Alternatively, lower inflammatory levels may simply accompany overall functional improvement. Because the present study lacked a parallel control group and did not systematically control for diet, sleep, physical activity, or background disease management, no direct causal effect of cognitive rehabilitation on inflammatory biology can be inferred from the current data.

From a translational perspective, the main value of this study lies in suggesting that variability in rehabilitation benefit should not be explained solely by training content or adherence. Biological background at the time of treatment entry may also matter. If future studies confirm these associations, blood-based inflammatory profiling may have potential value for baseline stratification before rehabilitation. At present, however, such markers should be viewed as exploratory rather than decision-defining tools. Patients expected to derive greater benefit could follow standard rehabilitation pathways, whereas those with high inflammatory burden might require longer duration, greater intensity, or multimodal strategies combining cognitive rehabilitation with exercise, sleep management, mood interventions, or targeted anti-inflammatory approaches.

Several limitations should be acknowledged. First, the self-controlled before–after design lacked a randomized control group, active comparator, or usual-care control arm, making it difficult to exclude the influence of spontaneous fluctuation, practice effects, placebo-related effects, caregiver involvement, and changes in daily routines. Repeated administration of cognitive tests such as the MMSE, MoCA, AVLT, DST, and TMT within a 12-week interval may also have inflated part of the observed improvement. Second, the sample size was modest, especially for biomarker reassessment, limiting the robustness of subgroup and dynamic analyses. Third, neuropsychiatric and behavioral variables, including depression, apathy, anxiety, sleep disturbance, and behavioral symptoms, were not systematically measured using validated scales. These factors are common in early AD and may influence both inflammatory status and cognitive performance. Fourth, although medication regimens were required to be stable before enrollment and during the intervention, detailed information on antidepressants, sedatives, antipsychotics, and other psychotropic medications was not fully incorporated into the regression models. Residual confounding by medication exposure therefore cannot be excluded. Fifth, only a limited set of peripheral immune-inflammatory biomarkers was measured. Peripheral IL-6, TNF-α, and CRP are nonspecific systemic markers, and GFAP may reflect astrocytic activation as well as broader neurodegenerative disease burden. Core blood biomarkers such as p-tau217, the Aβ42/Aβ40 ratio, and neurofilament light chain were not included, nor were PET or CSF data available to determine whether inflammatory status acts independently of core AD pathology. Future studies should adopt randomized controlled designs, larger samples, longer follow-up, repeated biomarker measurements, standardized assessment of neuropsychiatric symptoms, and integrated multi-marker panels.

In patients with early AD, 12 weeks of structured cognitive rehabilitation were associated with measurable improvements in global cognition, memory, executive function, and daily functioning. Higher baseline GFAP, IL-6, and TNF-α levels were associated with smaller cognitive and functional gains, with GFAP showing the most consistent association in the current dataset. Peripheral immune-inflammatory status may represent an exploratory biological correlate of rehabilitation response in early AD. These findings require further validation in randomized controlled studies with larger samples, longer follow-up, standardized neuropsychiatric assessment, and integrated AD-specific biomarker panels.

STATEMENT OF ETHICS

This study was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was reviewed and approved by the Ethics Committee of The Third People's Hospital of Chengdu. Written informed consent was obtained from all

participants or their legally authorized representatives prior to enrollment.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Not applicable.

DATA AVAILABILITY

The deidentified data supporting the findings of this study are available from the corresponding author, Jian Liu, upon reasonable request by email.

AI ASSISTANCE DISCLOSURE

Not applicable.

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