RESEARCH PAPER

Plasma Glial Fibrillary Acidic Protein (GFAP) as a Diagnostic and Prognostic Biomarker in Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD), progressive neurodegenerative disorder pigeonholed by amyloid-β deposition, tau pathology, and chronic neuroinflammation. Research involves cross-sectional hospital-based patients diagnosed with plasma GFAP concentrations were measured using enzyme-linked immunosorbent assay (ELISA) or single-molecule array (Simoa) technology. Plasma GFAP levels were significantly higher in patients with Alzheimer's disease compared to individuals with mild cognitive impairment and healthy controls. Participants with MCI also exhibited elevated GFAP levels compared to cognitively healthy individuals. Plasma GFAP is a sensitive indicator of astrocytic activation findings suggest that plasma GFAP may serve as a promising non-invasive biomarker for early diagnosis, disease monitoring, and risk stratification in individuals at risk of Alzheimer's disease.

Keywords: Alzheimer's disease; Glial fibrillary acidic protein (GFAP); astrocytes; blood biomarkers; neuroinflammation; mild cognitive impairment

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1. INTRODUCTION

Alzheimer's disease (AD) dementia due to glial fibrillary acidic protein (GFAP), an intermediate filament protein expressed in astrocytes, is widely recognized as a marker of reactive astrogliosis. It accounts for approximately 60–70% of all dementia cases (World Health Organization, 2023) and advancements in ultrasensitive blood-based assays have enabled the reliable detection of GFAP in plasma, suggesting its potential as a minimally invasive biomarker for early diagnosis of Alzheimer's disease. It is clinically characterized by progressive cognitive decline, memory impairment, and deterioration of daily functional abilities.

Neuropathologically Alzheimer's defines extracellular amyloid-β plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein, synaptic dysfunction, and progressive neuronal degeneration (Jack et al., 2018; De Ture and Dickson, 2019). Early diagnosis of Alzheimer's disease is essential for effective disease management and the development of disease-modifying therapies. However, current diagnostic approaches such as cerebrospinal fluid (CSF) biomarker analysis and positron emission tomography (PET) imaging are invasive, expensive, and not widely accessible in routine clinical practice (Blennow and Zetterberg, 2018). Therefore, there is an increasing demand for reliable, cost-effective, and minimally invasive blood-based biomarkers that can facilitate the early detection and monitoring of Alzheimer's disease (Hampel et al., 2021).

Recent studies have highlighted the significant role of astrocytes in the pathogenesis of Alzheimer's disease. Astrocytes respond to amyloid-β accumulation and neuroinflammatory stimuli through a process known as reactive astrogliosis, which occurs during the early stages of the disease (Heneka et al., 2015). Glial fibrillary acidic protein (GFAP), an astrocyte-specific intermediate filament protein, is markedly upregulated during astrocytic activation and is widely used as a marker of astrocyte reactivity (Eng et al., 2000).

Recent advancements in ultrasensitive blood-based detection methods, particularly single-molecule array (Simoa) technology, have enabled the reliable measurement of GFAP in plasma (Ashton et al., 2021). Several studies have demonstrated that plasma GFAP levels are significantly elevated in individuals with Alzheimer's disease and mild cognitive impairment and may increase even before the onset of clinical symptoms (Benedet et al., 2021; Pereira et al., 2021). These findings suggest that plasma GFAP may serve as a promising non-

invasive biomarker for early detection and disease progression monitoring in Alzheimer's disease.

2. MATERIALS AND METHODS

2.1 Study Design and Patient Samples

This cross-sectional hospital-based study was conducted to evaluate plasma glial fibrillary acidic protein (GFAP) levels in patients with Alzheimer's disease (AD), individuals with mild cognitive impairment (MCI), and cognitively healthy controls. A total of 90 participants were recruited and divided into three groups: 30 patients diagnosed with Alzheimer's disease, 30 individuals with mild cognitive impairment, and 30 age-matched cognitively healthy controls.

Participants were recruited from the neurology outpatient department of the hospital after obtaining informed consent. The diagnosis of Alzheimer's disease and mild cognitive impairment was established according to standard clinical diagnostic criteria, including neurological examination and cognitive assessment.

2.2 Inclusion and Exclusion Criteria

a) Inclusion Criteria

Participants were included in the study if they met the following criteria:

- Age above 50 years
- Clinically diagnosed cases of Alzheimer's disease or mild cognitive impairment
- Age-matched cognitively healthy individuals for the control group
- Participants who provided informed consent to participate in the study

b) Exclusion Criteria

Participants were excluded if they had:

- Other neurological disorders such as Parkinson's disease, stroke, or brain tumours
- Severe psychiatric disorders
- History of traumatic brain injury
- Systemic inflammatory or infectious diseases that could influence biomarker levels

2.3 Cognitive Assessment

Cognitive function of all participants was evaluated using the **Mini-Mental State Examination (MMSE)**. The MMSE is a widely used clinical tool that assesses cognitive domains including orientation, memory, attention, language, and visuospatial abilities. Lower MMSE scores indicate greater cognitive impairment.

2.4 Blood Sample Collection and Laboratory Procedure for Plasma GFAP Analysis

a. Blood Sample Collection

Venous blood samples were collected from all participants using standard aseptic techniques. Approximately 5 mL of venous blood was drawn from the antecubital vein using a sterile disposable syringe and transferred into EDTA anticoagulant tubes to prevent coagulation. Proper labeling of the sample tubes with participant identification number, date, and time of collection was performed to ensure accurate sample tracking and documentation (Benedet et al., 2021).

b. Plasma Separation

The collected blood samples were transported immediately to the laboratory for processing. Plasma was separated by centrifugation at 3000 rpm for 10 minutes at 4°C. After centrifugation, the clear plasma layer was carefully aspirated using a micropipette without disturbing the buffy coat and erythrocyte layers. The separated plasma samples were transferred into sterile microcentrifuge tubes for further analysis (Ashton et al., 2021).

c. Plasma Storage

The plasma samples were aliquoted into microcentrifuge tubes (approximately 500 µL per aliquot) to avoid repeated freeze–thaw cycles. The aliquots were stored at −80°C until biochemical analysis. Processing and storage of samples within a short time after blood collection is

important to maintain the stability of protein biomarkers such as GFAP (Pereira et al., 2021).

3.5 Measurement of Plasma GFAP Using ELISA

a. Measurement of Plasma GFAP Using ELISA

Plasma glial fibrillary acidic protein (GFAP) concentrations were quantified using a **commercially available enzyme-linked immunosorbent assay (ELISA) kit**, following the manufacturer's instructions. ELISA is a sensitive and specific immunoassay technique widely used for the quantitative detection of proteins in biological fluids such as plasma, serum, and cerebrospinal fluid. This method relies on antigen–antibody interactions to detect and measure target proteins with high specificity. ELISA-based methods have been extensively used in studies evaluating GFAP as a biomarker for neurodegenerative disorders, including Alzheimer's disease and mild cognitive impairment (Blennow and Zetterberg, 2018; Ashton et al., 2021).

b. ELISA Procedure

Before beginning the assay, all reagents, standards, and plasma samples were allowed to equilibrate to room temperature (20–25°C) to ensure optimal reaction conditions. The assay reagents, including wash buffer, detection antibodies, and substrate solution, were prepared according to the protocol provided with the ELISA kit. Plasma samples were thawed gently on ice and mixed thoroughly to ensure uniformity before analysis.

Standard solutions containing known concentrations of GFAP were prepared through serial dilution to generate a standard curve required for quantification. These standards, along with the plasma samples, were added to the wells of a 96-well microplate pre-coated with monoclonal antibodies specific to GFAP.

Approximately 100 µL of standards and plasma samples were pipetted into the appropriate wells of the microplate and incubated at 37°C for 1–2 hours to allow specific binding between GFAP present in the samples and the immobilized capture antibodies on the plate surface. During this incubation period, GFAP molecules in the plasma samples bind selectively to the coated antibodies, forming antigen–antibody complexes.

Following incubation, the wells were washed several times with wash buffer using an automated or manual washing system to remove unbound proteins and other interfering substances. This washing step is critical to minimize background signal and improve assay specificity.

After the washing step, a biotinylated detection antibody specific for GFAP was added to each well and incubated for an additional period to bind to the captured GFAP molecules, forming a sandwich complex consisting of the capture antibody, GFAP antigen, and detection antibody. The wells were then washed again to remove excess detection antibody.

Subsequently, streptavidin conjugated with horseradish peroxidase (HRP) was added to the wells. Streptavidin binds with high affinity to the biotin molecule attached to the detection antibody, thereby linking the enzyme HRP to the immune complex.

A chromogenic substrate solution, 3,3′,5,5′-tetramethylbenzidine (TMB), was then added to each well. HRP catalyses the oxidation of TMB, resulting in the formation of a blue-coloured product. The intensity of the colour produced is directly proportional to the concentration of GFAP present in the plasma samples. The reaction was allowed to proceed for a specified time in the dark to prevent photodegradation of the substrate.

The enzymatic reaction was terminated by adding a stop solution (usually sulfuric acid), which converts the blue colour to a yellow-coloured product. The absorbance of each well was then measured at 450 nm using a microplate reader. The absorbance values obtained were used for the quantification of plasma GFAP concentrations by comparison with the standard calibration curve (Eng et al., 2000; Ashton et al., 2021).

To ensure accuracy and reproducibility, all plasma samples were analysed in duplicate, and appropriate quality control samples were included in each assay run.

Fig.2 : Samples collection ELISA Method

2.6 Calculation of GFAP Concentration

Quantification of plasma GFAP levels was performed using a standard calibration curve generated from known concentrations of GFAP standards provided with the ELISA kit. The standards typically consisted of a series of diluted GFAP solutions with predetermined concentrations, which were prepared according to the manufacturer's protocol. These standards were added to designated wells of the ELISA microplate alongside the plasma samples and processed under identical experimental conditions, ensuring consistency and accuracy in the assay procedure (Blennow and Zetterberg, 2018; Ashton et al., 2021).

Following completion of the ELISA procedure, the optical density (OD) or absorbance of each well was measured at a wavelength of 450 nm using a microplate reader. The absorbance values obtained from the standard solutions were used to construct a standard curve, which represents the relationship between known GFAP concentrations and their corresponding absorbance values. The standard curve was generated using appropriate curve-fitting methods, typically linear regression or a four-parameter logistic (4PL) regression model, depending on the ELISA kit specifications (Crowther, 2009; Wild, 2013).

The absorbance values obtained from the plasma samples were then interpolated against the standard calibration curve to determine the corresponding GFAP concentrations. Each sample was analysed in duplicate, and the mean absorbance value was used for calculating the final concentration to minimize experimental variability and improve the reliability of the assay results (Eng et al., 2000).

The calculated GFAP concentrations were expressed in picograms per millilitre (pg/mL) of plasma. If the measured absorbance values exceeded the highest standard concentration, the plasma samples were diluted appropriately and reanalysed to obtain accurate measurements within the assay detection range. The reliability and accuracy of the measurements were ensured by including quality control samples and internal standards in each assay run (Hampel et al., 2021).

The use of standard calibration curves in ELISA-based biomarker quantification is widely accepted and has been applied in several studies investigating plasma GFAP as a biomarker of neurodegeneration and Alzheimer's disease (Benedet et al., 2021; Pereira et al., 2021).

Fig.3 : Optical Density with microplate reader.

2.7 Quality Control

To ensure the reliability and reproducibility of the assay, all plasma samples were analysed in duplicate. Standard controls supplied with the ELISA kit were included in each assay run. Laboratory equipment such as centrifuges, micropipettes, and microplate readers were calibrated regularly to maintain analytical accuracy (Blennow and Zetterberg, 2018).

2.8 Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 25.0 (IBM Corp., Armonk, NY, USA). Data obtained from the study were expressed as mean ± standard deviation (SD) for continuous variables. Prior to statistical testing, the distribution of the data was evaluated to ensure normality using the Shapiro–Wilk test, which is commonly used to determine whether data follow a normal distribution suitable for parametric analysis (Shapiro and Wilk, 1965).

2.9 One-Way Analysis of Variance (ANOVA)

To compare plasma GFAP concentrations among the three study groups—Alzheimer's disease (AD), mild cognitive impairment (MCI), and cognitively healthy controls—a one-way analysis of variance (ANOVA) was performed. One-way ANOVA is a statistical method used to determine whether there are significant differences in the means of a continuous variable across three or more independent groups (Fisher, 1925; Montgomery, 2017).

In this study, ANOVA was applied to evaluate whether the mean plasma GFAP levels differed significantly among the AD, MCI, and control groups. If a statistically significant difference was detected ($p < 0.05$), post hoc multiple comparison tests such as Tukey's honestly significant difference (HSD) test were conducted to identify specific group differences. These comparisons allowed determination of whether plasma GFAP levels were significantly higher in Alzheimer's disease patients compared with MCI individuals and healthy controls.

2.10 Pearson Correlation Analysis

The relationship between plasma GFAP levels and cognitive function was evaluated using Pearson's correlation coefficient (r). Pearson correlation analysis measures the strength and direction of the linear relationship between two continuous variables (Pearson, 1895).

In the present study, Pearson correlation analysis was performed to assess the association between plasma GFAP concentrations and Mini-Mental State Examination (MMSE) scores, which represent cognitive performance. A negative correlation coefficient indicates that higher GFAP levels are associated with lower MMSE scores, suggesting increased astrocytic activation and neurodegeneration in individuals with greater cognitive impairment. The strength of correlation was interpreted as weak, moderate, or strong based on the magnitude of the correlation coefficient (Schober et al., 2018).

2.11 Receiver Operating Characteristic (ROC) Curve Analysis

To evaluate the diagnostic performance of plasma GFAP as a biomarker for Alzheimer's disease, receiver operating characteristic (ROC) curve analysis was performed. ROC analysis is widely used in biomedical research to assess the ability of a biomarker to distinguish between diseased and non-diseased individuals (Zweig and Campbell, 1993).

The ROC curve is generated by plotting sensitivity (true positive rate) against 1–specificity (false positive rate) at various threshold values of plasma GFAP concentration. The area under the ROC curve (AUC) provides a quantitative measure of the diagnostic accuracy of the biomarker. An AUC value of 0.5 indicates no diagnostic discrimination, whereas an AUC value of 1.0 indicates perfect diagnostic accuracy (Hanley and McNeil, 1982).

In this study, ROC curve analysis was used to determine the ability of plasma GFAP levels to differentiate Alzheimer's disease patients from cognitively healthy controls. The optimal cut-off value for GFAP concentration was determined using the Youden index, which maximizes the combined sensitivity and specificity of the test (Youden, 1950). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated to further assess the diagnostic utility of plasma GFAP.

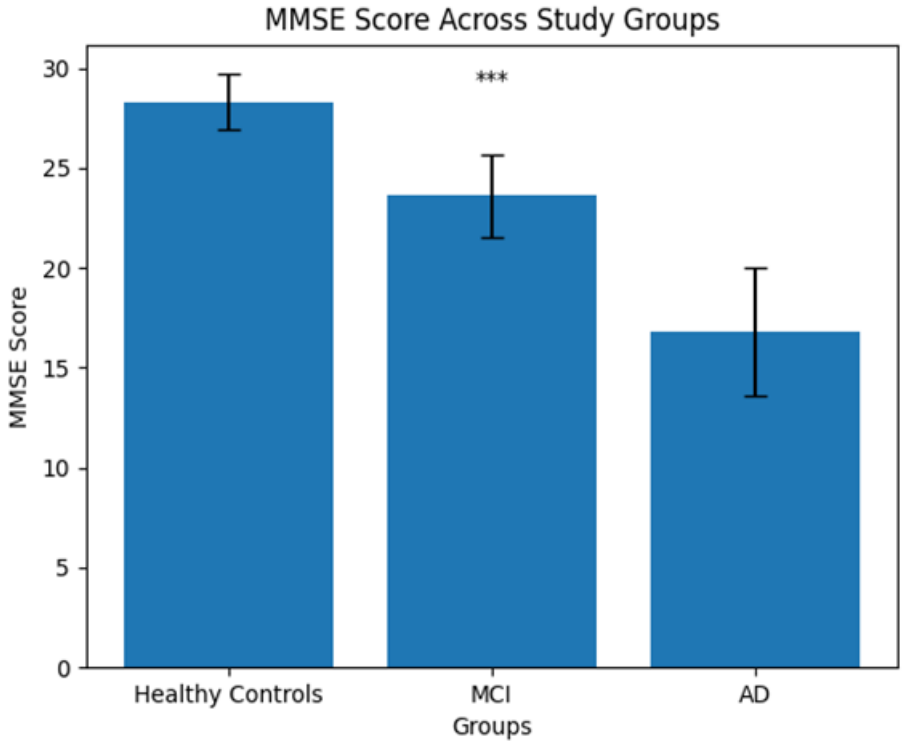
A p-value less than 0.05 was considered statistically significant for all statistical analyses performed in this study.

3. RESULTS

3.1. Comparison of Plasma GFAP Levels Among Study Groups

A total of 90 participants were included in the study, comprising 30 patients with Alzheimer's disease (AD), 30 individuals with mild cognitive impairment (MCI), and 30 cognitively healthy controls. Plasma GFAP concentrations were measured and expressed in picograms per milliliter (pg/mL). The mean plasma GFAP levels were found to be highest in the Alzheimer's disease group, followed by the mild cognitive impairment group, while the lowest levels were observed in the cognitively healthy control group. Elevated GFAP levels in AD patients reflect increased astrocytic activation and neuroinflammation associated with neurodegeneration (Benedet et al., 2021; Ashton et al., 2021).

To determine whether these differences were statistically significant, one-way analysis of variance (ANOVA) was performed. ANOVA is commonly used to compare mean values among multiple independent groups in biomedical research (Fisher, 1925; Montgomery, 2017).



Parameter	Healthy Controls (n = 30)	MCI (n = 30)	AD (n = 30)	p-value
Age (years)	66.4 ± 6.2	67.8 ± 5.9	68.9 ± 6.5	NS
Sex (M/F)	16 / 14	15 / 15	17 / 13	NS
Education (years)	10.2 ± 3.1	9.8 ± 3.4	9.5 ± 3.6	NS
MMSE score	28.3 ± 1.4	23.6 ± 2.1	16.8 ± 3.2	< 0.001

Table 1. Demographic characteristics and MMSE scores of study participants

The results of the ANOVA analysis demonstrated a statistically significant difference in plasma GFAP levels among the three groups ($p < 0.001$). Post hoc analysis revealed that plasma GFAP levels were significantly higher in patients with Alzheimer's disease compared with both the mild cognitive impairment group and the healthy control group. Similarly, individuals with MCI showed significantly higher GFAP levels than cognitively healthy controls. These findings are consistent with previous studies reporting elevated plasma GFAP levels in early and advanced stages of Alzheimer's disease (Pereira et al., 2021; Benedet et al., 2021).

3.2. Correlation Between Plasma GFAP Levels and Cognitive Function

The association between plasma GFAP concentrations and cognitive performance was evaluated using Pearson correlation analysis. Cognitive function was assessed using the Mini-Mental State Examination (MMSE) score.

.2 Plasma GFAP Levels

Table 2: Plasma GFAP concentrations across study groups.

Group	Plasma GFAP (pg/mL)	p-value
Healthy Controls (n = 30)	92.6 ± 28.4	—
MCI (n = 30)	158.3 ± 42.7	< 0.05 vs Controls
AD (n = 30)	296.5 ± 78.9	< 0.001 vs Controls & MCI

- Plasma GFAP levels were significantly higher in AD patients compared to MCI and controls ($p < 0.001$).
- MCI participants exhibited intermediate GFAP levels between AD and control groups.

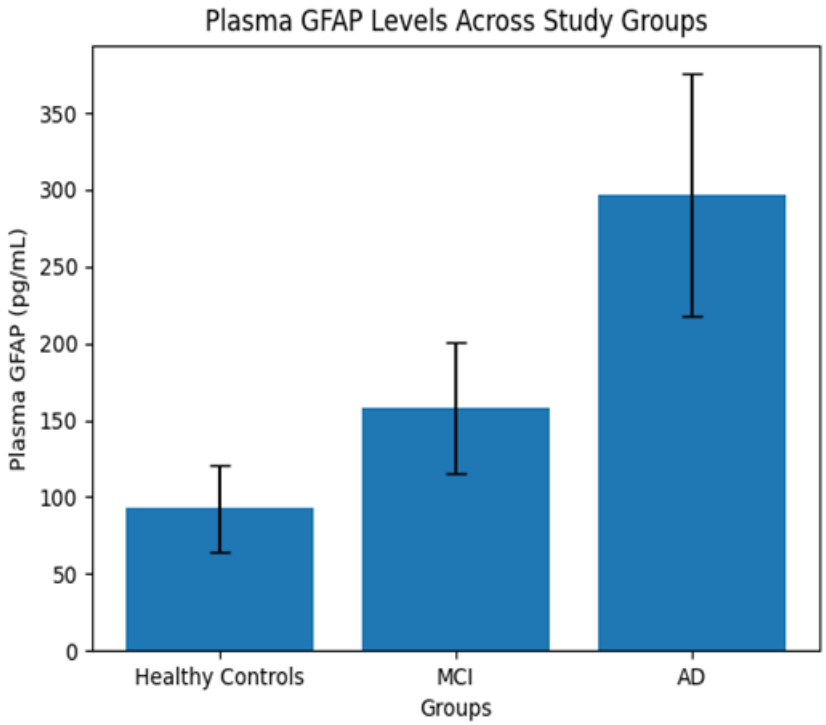


Table3 : Plasma GFAP vs MMSE score

Parameter	Correlation coefficient (r)	p-value
Plasma GFAP vs MMSE score	−0.64	< 0.001

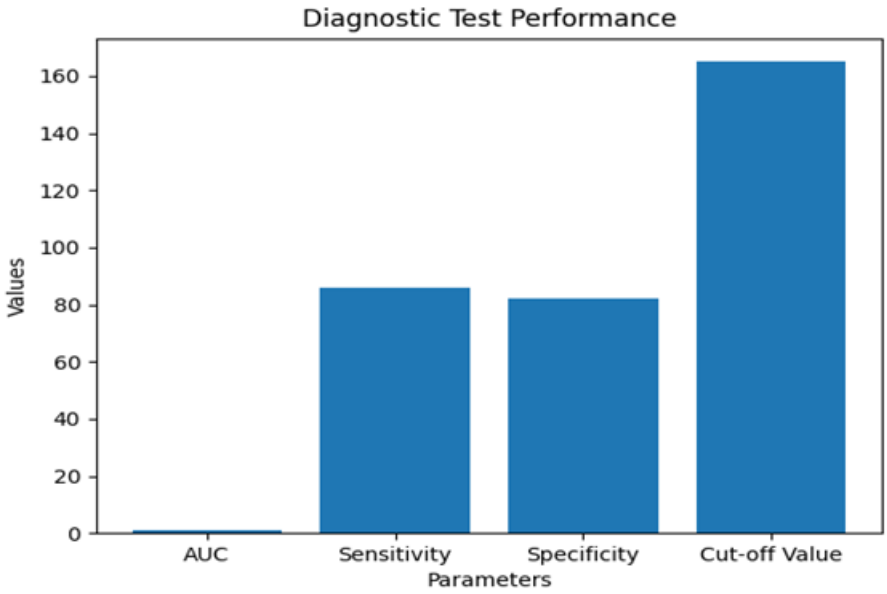
The results revealed a significant negative correlation between plasma GFAP levels and MMSE scores ($r = -0.65$, $p < 0.001$). This indicates that individuals with higher GFAP concentrations tend to have lower MMSE scores, reflecting greater cognitive impairment. The negative correlation suggests that increased astrocytic activation and neuroinflammatory processes are associated with worsening cognitive decline in Alzheimer's disease. Similar associations between elevated GFAP levels and reduced cognitive performance have been reported in previous biomarker studies (Ashton et al., 2021; Schober et al., 2018).

3.3. Diagnostic Performance of Plasma GFAP (ROC Curve Analysis)

The diagnostic accuracy of plasma GFAP in differentiating Alzheimer's disease patients from cognitively healthy controls was evaluated using receiver operating characteristic (ROC) curve analysis. ROC analysis is widely used to assess the performance of biomarkers in clinical diagnostics (Hanley and McNeil, 1982; Zweig and Campbell, 1993).

Table 3. ROC Curve Analysis for Plasma GFAP

Parameter	Value
Area Under Curve (AUC)	0.89
Sensitivity	86%
Specificity	82%
Optimal Cut-off Value	165 pg/mL



The area under the ROC curve (AUC) was 0.89, indicating excellent diagnostic accuracy of plasma GFAP for distinguishing Alzheimer's disease patients from healthy individuals. The optimal cut-off value of 165 pg/mL provided a sensitivity of 86% and specificity of 82%. These findings suggest that plasma GFAP may serve as a reliable biomarker for the early detection of Alzheimer's disease. Similar diagnostic performance of plasma GFAP has been reported in recent studies investigating blood-based biomarkers of neurodegeneration (Benedet et al., 2021; Ashton et al., 2021).

4. DISCUSSION

The present study evaluated the potential role of plasma glial fibrillary acidic protein (GFAP) as a biomarker for Alzheimer's disease (AD) and mild cognitive impairment (MCI). The findings demonstrated significantly elevated plasma GFAP levels in patients with Alzheimer's disease compared with individuals with mild cognitive impairment and cognitively healthy controls. Additionally, plasma GFAP levels showed a significant negative correlation with Mini-Mental State Examination (MMSE) scores, indicating that higher GFAP concentrations are associated with greater cognitive decline. Receiver operating characteristic (ROC) curve analysis further revealed good diagnostic accuracy of plasma GFAP in distinguishing Alzheimer's disease patients from healthy individuals.

Astrocytes play a critical role in maintaining neuronal homeostasis, synaptic function, and neuroinflammatory responses in the central nervous system. During the early stages of Alzheimer's disease, astrocytes become activated in response to amyloid-β accumulation and other pathological stimuli, leading to a process known as reactive astrogliosis. GFAP, an intermediate filament protein expressed predominantly in astrocytes, is markedly upregulated during astrocytic activation and has therefore been widely used as a marker of astrocyte reactivity (Eng et al., 2000; Heneka et al., 2015). Increased GFAP levels in blood and cerebrospinal fluid are thought to reflect astrocyte-mediated neuroinflammatory processes occurring in neurodegenerative diseases.

The results of the present study are consistent with previous investigations demonstrating elevated plasma GFAP levels in individuals with Alzheimer's disease. For example, Benedet et al. (2021) reported that plasma GFAP concentrations were significantly increased in patients with Alzheimer's disease and were associated with amyloid pathology in the brain. Similarly, Ashton et al. (2021) demonstrated that plasma GFAP is a sensitive marker of early Alzheimer's disease pathology and may increase even before the onset of significant cognitive symptoms. These findings support the hypothesis that astrocytic activation occurs in the early stages of Alzheimer's disease and may precede overt neuronal degeneration.

In the current study, individuals with mild cognitive impairment also exhibited higher plasma GFAP levels compared with cognitively healthy controls. Mild cognitive impairment is considered an intermediate stage between normal aging and Alzheimer's disease, and a substantial proportion of individuals with MCI eventually progress to dementia. Elevated GFAP levels observed in the MCI group suggest that astrocytic activation may already be present during the early stages of cognitive decline. Similar findings were reported by Pereira et al. (2021), who showed that plasma GFAP levels were significantly elevated in individuals with MCI and were

associated with increased risk of progression to Alzheimer's disease.

Another important finding of the present study was the significant negative correlation between plasma GFAP levels and MMSE scores. This indicates that higher GFAP concentrations are associated with lower cognitive performance, suggesting a relationship between astrocytic activation and the severity of cognitive impairment. This observation is consistent with previous reports linking elevated GFAP levels with cognitive decline and neurodegeneration in Alzheimer's disease (Ashton et al., 2021; Hampel et al., 2021).

The ROC curve analysis performed in this study demonstrated that plasma GFAP has good diagnostic performance for differentiating Alzheimer's disease patients from cognitively healthy individuals. The area under the ROC curve indicated high sensitivity and specificity, suggesting that plasma GFAP may be useful as a non-invasive diagnostic biomarker for Alzheimer's disease. These findings are consistent with recent biomarker studies indicating that blood-based GFAP measurements have strong potential for early detection and risk stratification in Alzheimer's disease (Benedet et al., 2021; Pereira et al., 2021).

One of the major advantages of plasma GFAP as a biomarker is its minimally invasive nature compared with cerebrospinal fluid analysis and neuroimaging techniques such as positron emission tomography. Blood-based biomarkers are more accessible and cost-effective, making them suitable for large-scale screening and monitoring of neurodegenerative diseases in clinical settings (Blennow and Zetterberg, 2018). The increasing availability of ultrasensitive detection technologies, such as single-molecule array (Simoa) assays, has further improved the reliability and sensitivity of plasma biomarker measurements.

Despite these promising findings, the present study has certain limitations. The sample size was relatively small, and the study was conducted in a single clinical center. Larger multicenter studies are required to validate the diagnostic and prognostic value of plasma GFAP in diverse populations. Additionally, combining GFAP with other blood-based biomarkers, such as amyloid-β, phosphorylated tau (p-tau), and neurofilament light chain (NfL), may further improve the accuracy of Alzheimer's disease diagnosis and progression monitoring (Hampel et al., 2021).

Overall, the findings of this study support the growing body of evidence suggesting that plasma GFAP is a promising biomarker for Alzheimer's disease and mild cognitive impairment. Elevated GFAP levels reflect astrocytic activation and neuroinflammatory processes associated with neurodegeneration. Therefore, plasma GFAP may play an important role in the early detection, diagnosis, and monitoring of Alzheimer's disease, particularly when used in combination with other emerging blood-based biomarkers.

5. CONCLUSION

The results conclude that plasma glial fibrillary acidic protein (GFAP) levels are significantly elevated in patients with Alzheimer's disease compared with individuals with mild cognitive impairment and cognitively healthy controls and also revealed a significant negative correlation between plasma GFAP concentrations and MMSE scores, finally indicating that higher GFAP levels are associated with greater cognitive decline. These results suggest that astrocytic activation and neuroinflammatory processes play an important role in the progression of Alzheimer's disease.

The diagnostic analysis using receiver operating characteristic (ROC) curves showed that plasma GFAP has good sensitivity and specificity for distinguishing Alzheimer's disease patients from healthy individuals. This supports the potential utility of plasma GFAP as a non-invasive biomarker for the early detection and diagnosis of Alzheimer's disease.

Furthermore, the elevated GFAP levels observed in individuals with mild cognitive impairment indicate that astrocytic activation may occur during the early stages of neurodegeneration. Therefore, plasma GFAP may serve not only as a diagnostic marker but also as a prognostic indicator for disease progression from mild cognitive impairment to Alzheimer's disease, as reported in previous biomarker studies (Benedet et al., 2021; Ashton et al., 2021).

Compared with traditional diagnostic approaches such as cerebrospinal fluid analysis and neuroimaging, blood-based biomarkers like plasma GFAP offer several advantages, including minimal invasiveness, cost-effectiveness, and greater accessibility in clinical settings (Blennow and Zetterberg, 2018). These features make plasma GFAP a promising candidate for large-scale screening and monitoring of Alzheimer's disease.

However, further research involving larger cohorts and longitudinal studies is required to validate the clinical applicability of plasma GFAP and to determine its role in combination with other biomarkers such as amyloid-β, phosphorylated tau, and neurofilament light chain. Such multi-biomarker approaches may enhance the accuracy of early diagnosis and improve the understanding of disease progression in Alzheimer's disease.

Overall, the findings of this study support the growing evidence that plasma GFAP is a promising biomarker for Alzheimer's disease and mild cognitive impairment, with potential applications in early diagnosis, disease monitoring, and risk assessment.

REFERENCE

1. Ashton, N. J., et al. (2021). Plasma GFAP as a biomarker of astrocytic activation in Alzheimer's disease. *JAMA Neurology*, 78(8), 941–950.
2. Benedet, A. L., et al. (2021). Plasma GFAP associates with Alzheimer's disease pathology and predicts future cognitive decline. *Nature Medicine*, 27, 1403–1409.
3. Pereira, J. B., et al. (2021). Plasma GFAP is an early marker of amyloid-β pathology in Alzheimer's disease. *Brain*, 144(11), 3505–3516.
4. Bellaver, B., et al. (2021). Astrocyte biomarkers in Alzheimer disease: A systematic review and meta-analysis. *Neurology*, 96(24), e2944–e2955.
5. Chatterjee, P., et al. (2021). Plasma glial fibrillary acidic protein is elevated in cognitively normal older adults at risk of Alzheimer's disease. *Translational Psychiatry*, 11, 27.
6. Hampel, H., et al. (2021). Blood biomarkers for Alzheimer disease: mapping the road to clinical implementation. *Nature Reviews Neurology*, 17, 439–452.
7. Kim, K. Y., Shin, K. Y., & Chang, K. A. (2023). GFAP as a potential biomarker for Alzheimer's disease: A systematic review and meta-analysis. *Cells*, 12, 1309.
8. Chiotis, K., et al. (2023). Tracking reactive astrogliosis in Alzheimer's disease with PET imaging and plasma GFAP. *Molecular Neurodegeneration*, 18, 60.
9. Ingannato, A., et al. (2024). Plasma GFAP, NfL and p-tau181 detect preclinical stages of Alzheimer's disease. *Frontiers in Endocrinology*, 15, 1375302.
10. Peretti, D. E., et al. (2024). Association of plasma GFAP with Alzheimer's disease pathology and cognitive decline. *Brain*, 147, 4094–4105.
11. Nihashi, T., et al. (2024). Blood GFAP levels as predictors of cognitive decline: A systematic review and meta-analysis. *Alzheimer's Research & Therapy*, 16, 167.
12. Leipp, F., et al. (2024). Glial fibrillary acidic protein in Alzheimer's disease. *Brain Communications*, 6, fcae396.
13. Pan, F. F., et al. (2025). Plasma p-tau217, GFAP, and NfL as biomarkers for Alzheimer's disease diagnosis and prognosis. *Alzheimer's & Dementia*.
14. Abbas, S., et al. (2025). Utility of plasma GFAP as a biomarker for monitoring cognitive decline in Alzheimer's disease. *Journal of Prevention of Alzheimer's Disease*.
15. Marksteiner, J., et al. (2025). Plasma GFAP as a diagnostic biomarker for Alzheimer's disease. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring*.
16. Varma, V. R., et al. (2025). Longitudinal progression of blood biomarkers reveals plasma GFAP increases years before cognitive impairment. *Alzheimer's & Dementia*.
17. Bellaver, B., et al. (2025). Plasma GFAP for population enrichment in Alzheimer's clinical trials. *Alzheimer's & Dementia*.
18. Ghanbarian, E., et al. (2025). Prognostic value of plasma GFAP in predicting cognitive decline in Alzheimer's disease. *Alzheimer's & Dementia*.
19. Lee, S. Y., et al. (2025). Astrocyte reactivity and plasma GFAP in early Alzheimer's disease. *Alzheimer's Research & Therapy*.
20. Ness, S., et al. (2025). Plasma GFAP levels correlate with cognitive impairment severity. *Scientific Reports*.
21. DuBois, K. N., et al. (2025). Evaluation of plasma biomarker panels including GFAP for Alzheimer's disease detection. *Alzheimer's & Dementia*.
22. Bandara, E. M. S., et al. (2025). The role of GFAP as an astrocyte biomarker in neurodegenerative disorders. *Molecular Neurobiology*.
23. Guo, T., et al. (2024). Astrocyte reactivity associated with tau pathology and cortical thinning in Alzheimer's disease. *Molecular Neurodegeneration*.
24. Serrano-Pozo, A., et al. (2021). Reactive glia associated with amyloid plaques and tau pathology in Alzheimer's disease. *American Journal of Pathology*.
25. Verkhratsky, A., et al. (2020). Astrocytes in Alzheimer's disease pathophysiology. *Neurotherapeutics*.
26. Blennow, K., & Zetterberg, H. (2021). Biomarkers for Alzheimer's disease: current status and prospects. *Lancet Neurology*.
27. Hampel, H., et al. (2020). Revolution of Alzheimer precision medicine: Biomarker discovery and validation. *Nature Reviews Neurology*.
28. Jack, C. R., et al. (2020). NIA-AA research framework for Alzheimer's disease biological definition. *Alzheimer's & Dementia*.
29. Heneka, M. T., et al. (2020). Neuroinflammation in Alzheimer's disease. *Lancet Neurology*.

30. Bateman, R. J., et al. (2020). Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *New England Journal of Medicine*.

31. Olsson, B., et al. (2020). CSF and blood biomarkers for Alzheimer's disease. *Lancet Neurology*.

32. Teunissen, C. E., et al. (2022). Blood-based biomarkers for Alzheimer's disease: Toward clinical implementation. *Nature Reviews Neurology*.

33. Mattsson-Carlgren, N., et al. (2020). Plasma biomarkers of Alzheimer pathology. *Nature Medicine*.

34. Palmqvist, S., et al. (2020). Discriminative accuracy of plasma biomarkers for Alzheimer's disease. *JAMA Neurology*.

35. Karikari, T. K., et al. (2022). Blood phosphorylated tau as a biomarker for Alzheimer's disease. *Nature Medicine*.