Plasma Glial Fibrillary Acidic Protein and Neurofilament-Light for the Diagnostic and Prognostic Evaluation of Frontotemporal Dementia

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Abstract

Background: Astrocytes play an essential role in neuroinflammation and are involved in the pathogenesis of neurodegenerative diseases. The Study of Glial fibrillary acidic protein (GFAP), an astrocytic damage marker, may be helpful to better understand the different neurodegenerative diseases. We investigated the diagnostic performance of plasma GFAP (pGFAP), plasma neurofilament (pNfL) and their combination in frontotemporal dementia (FTD) and Alzheimer's disease (AD). We studied their clinical utility in predicting disease progression.

Methods: We measured pGFAP and pNfL concentrations in 72 FTD, 56 AD and 83 cognitively normal participants (CN) using Single Molecule Array technology. Of 211 participants, 199 had CSF and 122 had MRI. We compared cross-sectional biomarker levels between groups, studied their diagnostic performance and assessed correlation with CSF biomarkers, cognitive performance and cortical thickness. The prognostic performance was investigated analyzing cognitive decline between group comparisons by tertile.

Results: Unlike pNfL, which was increased similarly in both clinical groups, pGFAP was increased in FTD but lower than in AD (all $p < 0.01$). The combination of both plasma markers improved the diagnostic performance to discriminate FTD from AD (combination AUC 0.78; pGFAP AUC 0.7; pNfL AUC 0.61, all $p < 0.05$). In FTD, pGFAP correlated with cognition, CSF and plasma NfL, and cortical thickness (all $p < 0.05$). The higher tertile of pGFAP was associated with greater change in MMSE score and poor cognitive outcome during follow-up both in FTD (1.40 points annually, HR 3.82, $p < 0.005$) and AD (1.20 points annually, HR 2.26, $p < 0.005$).

Conclusions: pGFAP and pNfL differed in FTD and AD, their combination could be useful to distinguish the two diseases. pGFAP could also be used to track disease severity and predict greater cognitive decline during follow-up in patients with FTD.

Background

Frontotemporal dementia (FTD) is a progressive neurodegenerative condition characterized by clinical, genetic and neuropathologic heterogeneity. The clinical manifestation of FTD may overlap with psychiatric or other neurodegenerative disorders, such as Alzheimer's disease (AD). Diagnosis is thus a clinical challenge.

In the past two decades, many efforts have been made to find imaging or fluid biomarkers for FTD¹⁻⁶. In spite of the notable advances, we still do not have pathophysiological markers of FTD to be used in clinical practice. Recently, some studies have shown that concentrations of NfL, a marker of axonal damage, are increased in CSF and blood in FTD patients. Moreover, their levels reflect disease severity and predict clinical progression¹⁻⁶. However, NfL is not specific of FTD and can be increased in other neurodegenerative diseases, such as AD or Lewy body dementia. There is considerable overlap in NfL levels between the different conditions¹⁻⁶.

Neuroinflammation and neurodegeneration are highly interrelated processes in neurological diseases⁷. Increased levels of Glial Fibrillary acidic protein (GFAP) in CSF and plasma, a marker of astrogliosis, have been described in different neurodegenerative diseases⁸⁻¹⁷. In AD, recent studies also showed that plasma GFAP (pGFAP) levels are associated with amyloid pathology. Furthermore, some studies have suggested that GFAP could be a marker of disease severity⁸⁻²¹ and be a prognostic marker for progression to AD dementia in cognitively normal older adults¹⁵⁻²¹. Likewise, other studies reported increased CSF and pGFAP concentrations in FTD, even though the results are not consistent across studies^{10, 12, 13, 22-26}. Although previous studies did not detect blood GFAP level changes in FTD^{10, 12, 13}, some studies observed elevated pGFAP in all different FTD subgroups²³⁻²⁶. Higher pGFAP concentrations have been associated with greater functional impairment and disease severity in two studies^{13, 24}, although the role of pGFAP in predicting disease progression in FTD and its potential use for diagnosis alone or in combination with other biomarkers remains unclear.

In this study, our primary research aim was to determine the potential use of pGFAP, pNfL or their combination to distinguish patients with FTD from AD and from cognitively normal participants (CN), and to study their association with disease progression in FTD. As a secondary objective we aimed to study the correlation of pGFAP with cognition, other biomarkers and structural measures in neuroimaging.

Methods

Study participants and classification. We collected clinical and biomarker information of 211 participants with available plasma samples from the Sant Pau Initiative on Neurodegeneration (SPIN) cohort, a multimodal biomarker platform for the study of neurodegenerative diseases²⁷. Participants were classified into one of the following clinical groups according to internationally accepted diagnostic criteria: 72 patients with probable FTD-related clinical syndromes and negative AD biomarkers: 33 patients with behavioral variant of frontotemporal dementia (bvFTD)²⁸, 7 semantic variant of primary progressive aphasia (svPPA)²⁹, 14 nonfluent variant of primary progressive aphasia (nfvPPA)²⁹ and 18 progressive supranuclear palsy-corticobasal syndrome spectrum (PSP-CBD)^{30, 31}. We also included 56 patients with Alzheimer disease (AD) with evidence of the AD pathophysiological process^{32, 33} either through CSF biomarkers ($n=51$) or amyloid-PET ($n=5$) and 83 CN that had normal CSF values of $A\beta_{42}/A\beta_{40}$, pTau181 and neuropsychological evaluation within normal range²⁷.

Plasma availability was the prerequisite to include the participants in the study. CSF samples were available in 199 participants (94%). A subset of 122 participants (58%) had undergone 3 Tesla structural brain MRI. *APOE* genotype was available in 203 participants (96%). 202 participants (96%) were longitudinally followed up and underwent a comprehensive evaluation. All participants had a Mini-mental state examination (MMSE) and a Global Deterioration Scale of Reisberg (GDS) score at the time of diagnosis, and repeated measures of MMSE were obtained during the follow-up. In a subset of 45 FTD patients, Frontotemporal Dementia Rating Scale (FTD-FRS) was available at the time of diagnosis.

Blood and CSF sample analysis. Blood was collected in 10ml EDTA tubes and immediately transferred to our laboratory where they were centrifuged and aliquoted within 2 hours after extraction. CSF samples were collected on the same day of blood extraction and processed in polypropylene tubes following international recommendations³⁴. All samples were processed and aliquoted within the first two hours after the lumbar puncture. Plasma and CSF aliquots were stored at -80°C until analysis. pGFAP and pNfL concentrations were measured using SR-X single molecule array (SIMOA). CSF AD core biomarkers (Aβ42, Aβ40, tTau and pTau181) were measured in the fully-automated platform Lumipulse (Fujirebio-Europe), and levels of CSF NfL (Uman Diagnostics) and CSF YKL-40 (MicroVue™, Quidel) were measured through ELISA using previously reported methods^{32,33}. The preanalytical protocol for blood and CSF in the SPIN cohort has been described in detail previously²⁷.

Statistical analysis. Chi-squared's test was used to compare sex and *APOE* status frequencies between groups. Continuous variables were expressed as means and standard deviation (SD). Distributions for demographic and biomarker data were assessed using Shapiro-Wilk's test and homogeneity of variances was checked by Levene's test. Biomarker raw values not following a normal distribution were log-transformed to achieve a normal distribution. Age- and sex-adjusted analysis of covariance (ANCOVA) followed by *post-hoc* Tukey test was used to compare pGFAP and pNfL levels between groups. To investigate the potential use of pGFAP, pNfL or their combination to distinguish patients with FTD from AD and CN, we assessed areas under the curve (AUC) by receiver operating characteristic (ROC) analyses. ROC curves were compared using DeLong's test. To study the association of pGFAP with disease progression, we divided participants in 3 tertile groups according to their pGFAP levels. A linear-mixed model was used to assess the association of pGFAP with cognitive decline during the follow-up. We included age, sex, MMSE score at the time of diagnosis, pGFAP tertile and its interaction with time as fixed factors, and modeled random intercepts and slopes at the participant level to account for repeated measures. Multivariate Cox regression analysis adjusting for sex and baseline age and Kaplan Meier curves were performed to analyze the predictive value of pGFAP for significant cognitive impairment (MMSE score <20). In order to investigate the relationship of pGFAP concentrations with demographics, cognitive scores, CSF biomarkers and pNfL concentrations, we assessed Spearman's correlations. Cortical thickness was computed with Freesurfer software³⁵ (version 5.1) (<https://surfer.nmr.mgh.harvard.edu/fswiki>), and its correlation with pGFAP was assessed through general linear model, including age and sex as fixed factors.

Statistical significance for all tests was set at 5% (p=0.05) and corrected for multiple comparisons. All the statistical analyses were performed using packages "psych" (v. 2.0.8), "ggplot2" (v. 3.3.3), "pROC" (v. 1.16.2), "lmerTest" (v. 3.1-3), "nlme" (v. 3.1-148), "multcomp" (v. 1.4-13), "survival" (v. 3.2-11) and "survminer" (v. 0.4.9) as implemented in R statistical software version R 4.0.2 (<http://www.R-project.org>).

Results

Demographics and clinical data

Table 1 shows demographics and clinical data, CSF and plasma biomarker levels in the FTD, AD and CN groups. Controls were significantly younger than symptomatic patients, but there was no difference in age between the symptomatic groups. Controls had more years of education than the symptomatic groups, and there were no differences in sex between the groups. *APOE*ε4 genotype was more frequent in the AD group. Both baseline and last MMSE scores were lower in disease groups compared with controls. Within FTD subgroups, baseline and last MMSE scores were lower in svPPA compared to other subgroups. The mean follow-up period was 3.4 (±2.3) years in FTD, 3.9 (±2) in AD and 4 (±1.7) in CN. Follow-up period did not differ between the groups.

Age correlated with pGFAP (Rho 0.35, p<0.001) concentrations in the control group, but not with pNfL levels. No difference in pGFAP concentration was found between male and female participants. pNfL levels were higher in male in the whole sample (p=0.05), and in the AD group (p=0.04), but not in CN or in the FTD groups.

TABLE 1. Demographics and clinical characteristics, CSF and plasma biomarkers concentrations in CN, FTD and AD

	CN	FTD					AD
		bvFTD	nfvPPA	svPPA	PSP-CBD	All FTD	
N	83	33	14	7	18	72	56
Age, years	58(8.5) ^{b, c}	68.3(10.6) ^a	72.8(5.6) ^a	73.1(11.1) ^a	72.9(5.4) ^a	70.8(8.9) ^a	70.8(7.2) ^a
Sex							
Female	49	10	9	4	11	34	31
Male	34	23	5	3	7	38	25
APOE ε4 status							
ε4 +	23 ^c	3 ^c	3 ^c	2 ^c	2 ^c	10 ^c	27 ^{a, b, d-g}
ε4 -	57 ^c	29 ^c	11 ^c	5 ^c	14 ^c	59 ^c	27 ^{a, b, d-g}
Follow up time, years	3.9 (1.7)	4.1(2.5)	3.1(2.3)	4.8(1.7)	1.9(1)	3.4(2.3)	4(2)
Education, years	15.4(4.1) ^{b-g}	12.9(5.6) ^a	13.3(4.9) ^a	14.8(5.2) ^a	11.3(3.7) ^a	12.7(5) ^a	10.4(4.7) ^a
Baseline MMSE score	29.2(1) ^{b-g}	25.1 (4.2) ^{a, f}	24.1(6.8) ^{a, f}	16.5(12.2) ^{a, c-e, g}	22.6(6.3) ^{a, f}	23.5(6.5) ^a	23.7(3.8) ^a
Last MMSE score	29.3 (1) ^{b-g}	16.6(10) ^{a, F}	14(12.2) ^{a, f}	0.4 (0.9) ^{a, c-e, g}	11.6(12.4) ^a	13.3(11.4) ^a	15.5(9.5) ^a
pGFAP, pg/ml	134.3(45.4) ^{b-d, f-g}	193.1(100.1) ^a	224.6(77.2)	159.3(279.1) ^a	271.2(148.2) ^a	234.9(141.9) ^{a, b}	319.8 (135.1) ^{a, c}
pNfL, pg/ml	18(20.2) ^{b-e, g}	41.9(60.1) ^a	34.9(17.2) ^a	31.3(14.5)	34.3(18.1) ^a	37.6(42.3) ^a	26.5(13.3) ^a
CSF NfL, pg/ml	494.8(274.3) ^{b, c, f, g}	1436.3(930.6) ^a	1944.5(1146.7)	2394.6(637.8) ^a	1739.3 (1607.3) ^a	1654.1(1187.6) ^{a, c}	1286.4(1136.9) ^{a, b, f}
CSF YKL-40, pg/ml	190.3(49.4) ^{b, c}	265.8(64.7) ^a	274.84(61) ^a	311.8(37.1) ^a	262.3(79.3)	268.2(63.7) ^a	287.3(68.1) ^a
<p>Unless otherwise specified, values are expressed as mean (standard deviation).</p> <p>Abbreviations: MMSE = Mini-Mental State Examination; pGFAP = plasma Glial fibrillary acidic protein; pNfL = plasma Neurofilament; CN = cognitively normal participants; FTD = Frontotemporal dementia; AD = Alzheimer Disease; bvFTD = behavioral variant of frontotemporal dementia; nfVPPA = nonfluent variant of primary progressive aphasia; svPPA = semantic variant of primary progressive aphasia; PSPCBD = progressive supranuclear palsy-corticobasal syndrome spectrum</p> <p>^aDifferent from Control (p<0.05); ^bDifferent from FTD (p<0.05); ^cDifferent from AD (p<0.05); ^dDifferent from bvFTD(p<0.05); ^eDifferent from nfVPPA(p<0.05); ^fDifferent from svPPA(p<0.05); ^gDifferent from PSPCBD(p<0.05). Data are shown as mean (standard deviation).</p>							

Plasma GFAP and NfL concentrations differ in FTD and AD

As shown in Figure 1, after adjusting by age and sex, pGFAP and pNfL concentration differed between FTD and AD patients.

Concentrations of pGFAP were higher in patients with FTD (234.9±141.9pg/ml) than in CN (134.3±45.4pg/ml, p=0.0008) but lower than in AD (319.8±135.1pg/ml, p<0.001). Within FTD subgroups, pGFAP concentrations were significantly higher in svPPA and PSP-CBD compared with controls (p=0.03), and concentrations in bvFTD were lower than in AD (p<0.001) (Figure 1 A and B).

As expected, pNfL concentrations were higher both in FTD (37.6±42.3 pg/ml, p<0.001) and AD (26.5±13.3pg/ml, p=0.004) groups compared to CN (18±20.2pg/ml). pNfL concentrations were increased in all FTD subgroups (p<0.05) compared to CN, except svPPA, which showed a similar trends than the rest of the FTD subgroups but did not reach significance (Figure 1C and D).

Diagnostic accuracy of plasma biomarkers and their combination to differentiate between FTD, AD and CN

We next explored the diagnostic performance of pGFAP, pNfL and their combination using logistic regression to discriminate across the different groups. The basic model included age, sex and *APOEε4* allele status. We compared the diagnostic performance of this basic model with a panel including the variables in the basic model together with pGFAP and pNfL.

A ROC curve analysis is shown in Figure 2. The combination of pGFAP and pNfL showed higher accuracy than the two plasma markers separately to differentiate FTD from AD (combination: AUC 0.78 [95%CI 0.70-0.86]; pGFAP: AUC 0.7 [95%CI 0.61-0.71]; pNfL: AUC 0.61 [95%CI 0.51-0.71], $p=0.04$ and $p=0.002$, respectively) and for the discrimination between FTD and CN (Combination: AUC 0.82 [95%CI 0.75-0.89]; pGFAP: AUC 0.76 [95%CI 0.68-0.84]; pNfL: AUC 0.81 [95%CI 0.74-0.88], $p=0.02$ and $p=0.51$, respectively). None of the two plasma markers, individually or in combination, showed higher accuracy than the basic model. However, the addition of pGFAP and pNfL to the basic model (panel model) showed higher diagnostic performance that outperformed the basic model both in differentiating FTD from AD (AUC 0.83 [95%CI 0.75-0.9] vs AUC 0.69 [95%CI 0.6-0.79], $p=0.003$) and AD from CN (AUC 0.96 [95%CI 0.93-1] vs AUC 0.89 [95%CI 0.83-0.95], $p=0.0007$). To differentiate FTD from CN, the panel showed highest AUC compared to other methods (all $p<0.05$), but not compared to the basic model (AUC 0.88 [95%CI 0.83-0.94] vs AUC 0.87 [95%CI 0.81-0.93], $p=0.08$).

Plasma GFAP correlated with other fluid biomarkers, cognitive and functional scores and neuroimaging

In the whole sample, pGFAP concentrations correlated with pNfL ($\rho=0.53$, $p<0.001$), CSF NfL ($\rho=0.52$, $p<0.001$) and CSF YKL-40 concentrations ($\rho=0.49$, $p<0.001$) after adjusting by age and sex. To avoid group effects in the correlation assessment, we studied correlations of these biomarkers within groups. pGFAP concentrations correlated with plasma and CSF NfL in FTD ($\rho=0.49$, $p<0.001$; $\rho=0.32$, $p=0.02$) and in CN ($\rho=0.4$, $p<0.001$; $\rho=0.3$, $p=0.016$), but not with CSF YKL-40. By contrast, in the AD group, pGFAP correlated with pNfL only ($\rho=0.35$, $p=0.007$) (Figure 3A-C).

After adjusting by age and sex, pGFAP was also significantly associated with baseline MMSE scores in the whole sample ($\rho=-0.58$, $p<0.001$) and in the FTD group ($\rho=-0.33$, $p=0.005$), but not in AD or in the CN groups (Figure 3D). In the FTD group, after adjusting by age, higher pGFAP concentrations were associated with lower FTD-FRS scores ($r=-0.28$, $p=0.046$, Figure 3E).

We studied the correlation between pGFAP and cortical thickness in a subset of 122 participants (29 FTD, 25 AD and 68 CN) with structural MRI suitable for quantitative analyses. After adjusting by age, sex and neuroimaging acquisition center, a vertex-wise regression analysis showed a significant association between pGFAP and cortical thickness in the orbitofrontal and occipital pole regions (Figure 3F, $p<0.05$) in the FTD group. In the AD and the CN groups, no correlation between pGFAP concentration and cortical thickness was found (not shown).

Baseline plasma GFAP levels predict cognitive decline

To investigate the prognostic performance of pGFAP levels, we divided pGFAP levels in tertiles within each symptomatic group: low (<154 pg/ml in FTD and <228 pg/ml in AD), medium (154-240pg/ml in FTD and 228-366pg/ml in AD) and high (>240 pg/ml in FTD and >366 pg/ml in AD).

A linear-mixed model analysis was used to assess the relationship between baseline pGFAP concentration and cognitive decline measured by change in MMSE score during follow-up. After adjusting by age, sex and baseline MMSE score, patients in the highest pGFAP tertile showed a greater change in MMSE compared to those in the lowest tertile in both FTD (mean loss of 1.40 points annually, $p=0.003$) and in AD (mean loss of 1.20 points annually, $p<0.001$) (Figure 4 A and B).

Kaplan Meier curves (Figure 5) and Cox regression analyses including baseline age and sex as covariates were used to assess progression to moderate cognitive impairment (MMSE score < 20). Compared to the lowest tertile, the highest tertile of pGFAP was associated with increased risk of poor cognitive outcome both in FTD and in AD (1.40 points annually, HR 3.82 in FTD; 1.20 points annually, HR 2.26 in AD, both $p<0.001$).

Discussion

In this study, we found that pGFAP is increased in FTD, and that its concentration differs from that in AD patients. pGFAP also correlated with neuronal damage biomarkers, cognitive and functional scores and with structural imaging measures in FTD. Importantly, a higher pGFAP concentration was associated with a greater change in MMSE score during follow-up and predicted progression to moderate cognitive impairment in FTD. Our findings suggest that pGFAP could be a useful marker of disease severity and prognostic assessment in FTD, in addition to its role in AD.

Emerging evidence suggests that astrocytes play an essential role in neuroinflammation and are involved in the pathophysiology of several neurological diseases^{34,37}. Biomarkers that track astrocyte biology, such as GFAP, have been investigated over the past few years in neurodegenerative diseases. In AD, previous studies have shown that reactive astrocytes are closely associated with senile plaques and neurofibrillary tangles³⁷⁻³⁹. Elevated concentrations of GFAP in CSF and blood have been observed in AD, and it has been reported that its levels correlate with disease severity^{8-16,18}. Recent studies have shown that elevated CSF and pGFAP concentration were associated with amyloid pathology, also in cognitively unimpaired subjects^{15-17,19}. In addition, astrogliosis is also recognized in FTD. Astrogliosis is mainly confined to concrete regions such as frontal cortex and the hippocampus^{40,41}. Interestingly, increased CSF and plasma GFAP concentrations have been reported both in sporadic and genetic FTD cohorts^{10-12,14,22}. These findings suggest that pGFAP may be a valuable tool in FTD. According to this hypothesis, various studies observed elevated pGFAP concentration in different FTD subgroups and both in presenile and late-onset bvFTD cases^{23,24,26}. In addition, one study in genetic FTDs found increased pGFAP concentration in progranulin-associated symptomatic FTD patients²². In agreement with previous studies, here we report an increased pGFAP concentration in FTD patients but to a lesser degree than that of AD. This result supports previous clinical-pathologic findings that show in FTD, there is less astrocytic activation and it is more confined to specific brain regions than in AD⁴⁰.

⁴¹. On the other hand, the increase in pNfL, a marker of axonal damage, suggests a similar degree of neurodegeneration in FTD compared to AD. One possible explanation for the differences found in pGFAP levels but not in pNfL is that FTD and AD are characterized by different neuroinflammatory patterns, with different degree of astroglia activation in different subpopulations but leading to a similar degree of neuronal degeneration. Similar to a prior study²⁴, we found that pGFAP concentration tended to be different across the subtypes of the FTD spectrum, being significantly higher in svFTD and PSP-CBD compared to CN. This finding could indicate differences in the degree of astrocytic degeneration in different clinical phenotypes.²².

The current study showed that pGFAP had an acceptable performance to discriminate FTD from AD (AUC=0.7) and CN (AUC=0.76). These accuracies were somewhat lower than the values reported in two recent studies using pGFAP to identify bvFTD from AD (AUC =0.85) and frontotemporal lobar degeneration (FTLD) from primary psychiatric disorders (AUC=0.82)^{13,26}. These discrepancies could be attributed to differences in cohort characteristics and heterogeneity due to different composition of FTD subtypes. An interesting finding was that, contrary to pNfL, which tend to be higher in FTD compared to AD, pGFAP levels were significantly lower in FTD, which suggests the potential diagnostic utility of their combination. Indeed, the combination of pGFAP with pNfL improved the diagnostic performance to distinguish FTD from AD and CN. Moreover, the incorporation of age, sex and *APOEε4* allele improved the diagnostic performance up to an AUC of 0.83 in differentiating FTD from AD and AUC of 0.89 in differentiating FTD from CN. In addition, pGFAP was particularly promising in identifying AD, in agreement with previous observations^{13,15,17-19}. Taken together, this study suggests that the combination of plasma astrocytic and neuronal markers could be relevant in FTD.

We observed that pGFAP concentration increased with age in controls, similarly to what has been observed in previous studies^{13,17,22,24}, and we took age into account when interpreting this marker. In agreement with various recent reports^{15,17,20,22}, pGFAP significantly correlated with CSF and plasma NfL, suggesting that glial activation and neuroaxonal degeneration are correlated. Previous studies have reported a weak correlation between CSF GFAP and YKL-40 levels in neurodegenerative diseases^{11,12}. Similarly, we did not find significant correlation between pGFAP and CSF YKL-40 levels. This finding supports the hypothesis that different astrocyte subpopulations or different spatial distribution are involved in the pathophysiology of FTD and AD^{12,37-41}. The association between elevated pGFAP concentration, disease severity and prognostic markers in AD has been reported before^{15,17-21}. Previous studies reported that serum GFAP had potential to predict future conversion to dementia in cognitively normal individuals, not only to AD, but also to other dementias including FTD^{16,17,21}. However, pGFAP as a marker of disease severity and prognosis in FTD has seldom been investigated^{13,24,25}. In the current study, after adjusting by age and sex, pGFAP remained significantly correlated with cognitive and imaging measures in FTD. Higher concentration of pGFAP have been shown to be associated with lower brain volumes in GRN and C9orf72 presymptomatic carriers²², and its association with smaller hippocampal volume in svPPA²⁵ and temporal atrophy in FTLD has been reported²⁶. To our knowledge this is the first study to show that higher concentration of pGFAP are also associated with orbitofrontal cortical thickness in the whole FTD group. In line with other reports in AD patients^{16,19,21}, we observed that pGFAP predicted faster cognitive decline both in FTD and AD. Association between pGFAP with cognitive decline in FTD had not been previously studied; our study shows a higher rate of cognitive decline among those with pGFAP >240pg/ml. Surprisingly, the relationship between pGFAP and disease severity in AD was not confirmed in our cohort. One of the considerations to take into account when comparing our study with the previous ones is that others included both Aβ+ and Aβ- subjects^{15,21}, whereas in our study the correlation analysis was performed in disease subgroups. Previous studies found that pGFAP were increased in early stages of AD¹⁵⁻²¹ and did not differ between disease stage¹⁸⁻¹⁹, which could explain the lack of association with imaging measures in the AD subgroup. In agreement with previous studies^{2,7,16,32}, our results show that glial biomarkers increase also in FTD besides AD, and its levels increase later in the disease course in FTD than in AD, when the disease is more advanced.

The strength of our study is the relatively large sample size, the inclusion of several FTD subgroups, regular follow-up of the subjects and multimodal approach, with inclusion of clinical measures, plasma, CSF and structural imaging. Additionally, all blood-based biomarkers were collected using the same standard operating procedures and measured following a harmonized protocol. This study has also some limitations. This study did not include serial longitudinal measures of pGFAP, the study lacks neuropathological confirmation, and we excluded AD copathologies in FTD groups.

Conclusion

In conclusion, our study shows that pGFAP concentration may be useful in FTD and could improve its diagnostic performance. Furthermore, our data support that pGFAP is not an exclusive marker in AD, but also play an essential role in other amyloid-independent processes involved in FTD. pGFAP could be useful as disease severity and prognostic marker in FTD.

Abbreviations

AD: Alzheimer's disease; AUC: Area under the curve; bvFTD: behavioral variant of Frontotemporal dementia; CN: cognitively normal participants; CSF: Cerebrospinal fluid; FTD: frontotemporal dementia; FTLD: frontotemporal lobar degeneration; FTD-FRS: Frontotemporal Dementia Rating Scale; GDS: Global Deterioration Scale of Reisberg; GFAP: Glial fibrillary acidic protein; HR: Hazard ratio; MMSE: Mini-Mental State Examination; NfL: neurofilament-light; nvPPA: nonfluent variant of primary progressive aphasia; PET: Positron emission tomography; pGFAP: plasma Glial fibrillary acidic protein; pNfL: plasma neurofilament-light; PSP-CBD: progressive supranuclear palsy-corticobasal syndrome spectrum; ROC: receiver operating

characteristic; SD: standard deviation; SIMOA: single molecule array; SPIN: Sant Pau Initiative on Neurodegeneration; svPPA: semantic variant of primary progressive aphasia.

Declarations

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Ethics approval and consent to participate

All procedures in the study were approved by the ethics committee at Hospital Sant Pau. All participants provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyses during the current study are available from the corresponding author on reasonable request.

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Authors’ contributions

NZ, DA and AL designed the study. NZ, MS, IIG, VM, TE, IB, MA, JA, LM, OB, IS, MBSS, AS, LV, RB, JC, MCI, JF, AL and DA acquired data relevant for the study. NZ, DA and VM performed statistical analysis. NZ, DA and AL contributed in analysis and interpretation of data. DA and AL participated in study supervision or coordination. NZ, DA, AL drafted the first version of the manuscript. All authors revised the manuscript for content and provided critical feedback.

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Figure 1

Comparison of pGFAP and pNfL in different groups: pGFAP in the FTD, AD and cognitively normal groups (A); pGFAP concentration in all FTD subgroups, AD and cognitively normal groups (B); pNfL concentration in the FTD, AD and cognitively normal groups (C). pNfL concentration in all FTD subgroups, AD and cognitively normal groups (D). * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Abbreviations: pGFAP = plasma Glial fibrillary acidic protein; pNfL = plasma Neurofilament light; CN = cognitively normal participants; FTD = Frontotemporal dementia; AD = Alzheimer Disease

Figure 2

Receiver operating characteristic curves (ROC) for plasma GFAP, plasma neurofilament light, their combination, a basic model with risk factors (age, sex and APOE) and a combination of plasma biomarkers with additional risk factors to assess the accuracy to discriminate FTD from AD (A), FTD from normal cognitively normal (B), and AD from cognitively normal (C). Abbreviations: AUC = area under the curve; pGFAP = plasma Glial fibrillary acidic protein; pNfL = plasma Neurofilament light; CN = cognitively normal participants; FTD = Frontotemporal dementia; AD = Alzheimer Disease

Figure 3

Correlation of pGFAP with plasma and CSF neurofilament light (A, B), CSF YLK (C), baseline MMSE score (D) in FTD, AD and normal cognitively. Correlation of plasma GFAP with baseline FTD-FRS score (E) and with cortical thickness in FTD (F, n=29). Blue regions represent a direct correlation. For illustrative purposes, an scatterplot shows the individual log (pGFAP) and the value of cortical thickness in the corresponding cortical region (marked with an asterisk) (F). P-value $10^{-1.3} = 0.05$. All the correlation analysis are adjusted by age and sex. pGFAP = plasma Glial fibrillary acidic protein; pNfL = plasma Neurofilament light; CN = cognitively normal participants; FTD = Frontotemporal dementia; AD = Alzheimer Disease

Figure 4

Relationship between baseline tertile of pGFAP and longitudinal changes of cognitive score (MMSE score) in FTD (A) and AD (B). Cognitive changes were estimated through linear mixed effects regression models adjusted for age and baseline MMSE score. Red lines show the lowest tertile (below 154pg/ml in FTD and below 228pg/ml in AD), green lines show medium tertile (154-240pg/ml in FTD and 226-366pg/ml in AD), and blue lines show the highest tertile of biomarker concentrations (above 240pg/ml in FTD and above 366pg/ml in AD). Shaded areas indicate the 95% confidence for predicted cognitive scores. Abbreviations: pGFAP = plasma Glial fibrillary acidic protein; pNfL = plasma Neurofilament light; CN = cognitively normal participants; FTD = Frontotemporal dementia; AD = Alzheimer Disease

Figure 5

Kaplan Meier curve of clinical progression to significant cognitive impairment (MMSE score<20) for individuals with low, medium or high baseline plasma GFAP in FTD (A) and in AD (B). Red lines show the lowest tertile (below 154pg/ml in FTD and below 228pg/ml in AD), green lines show medium tertile (154-240pg/ml in FTD and 226-366pg/ml in AD), and blue lines show the highest tertile of biomarker concentrations (above 240pg/ml in FTD and above 366pg/ml in AD). In FTD, the median time of progression to significant cognitive impairment was 6.4 (5-9) years in medium tertile pGFAP group and 2.4 (2.1-3.5) years in high tertile pGFAP group (Log-rank test, $p<0.0001$). In AD, the median time of progression to significant cognitive impairment was 5.7 (4.6-6.7) years in low tertile pGFAP group, 5.1 (3.5-6.6) years in medium tertile pGFAP group and 2.7 (2-3.7) years in high pGFAP tertile group (Log-rank test, $p=0.0002$). Abbreviations: pGFAP = plasma Glial fibrillary acidic protein; FTD = Frontotemporal dementia; AD = Alzheimer Disease