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Research paper

Serum glial fibrillary acidic protein and neurofilament light chain in treatment-naïve patients with unipolar depression☆

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ABSTRACT

Background: Unipolar depression has been associated with increased levels of glial dysfunction and neurodegeneration biomarkers, such as Glial Fibrillary Acidic Protein (GFAP) and Neurofilament light chain (NfL). However, previous studies were conducted on patients taking psychotropic medication and did not monitor longitudinal associations between disease status and GFAP/NfL.

Methods: Treatment-naïve patients with unipolar depression (n = 110) and healthy controls (n = 33) were included. GFAP/NfL serum levels were analyzed by Single Molecule Array at baseline and 3-month follow-up. The primary endpoint was GFAP/NfL levels in patients with depression compared with healthy controls. The secondary endpoint was the associations between GFAP/NfL with depression severity and cognitive function.

Results: The patients' mean HAM-D17 score was 18.9 (SD 3.9) at baseline and improved by 7.9 (SD 6.8) points during follow-up. GFAP/NfL was quantified in all individuals. At baseline, the adjusted GFAP levels were −16.8 % (95 % CI: −28.8 to −1.9, p = 0.03) lower among patients with depression compared to healthy controls, while NfL levels were comparable between the groups (p = 0.57). In patients with depression, mean NfL levels increased from baseline to follow-up (0.68 pg/ml, p = 0.03), while GFAP levels were unchanged (p = 0.24). We did not find consistent associations between NfL/GFAP with depression scores or cognitive function.

Conclusion: This largest study of serum NfL/GFAP levels in patients with depression did not support previous findings of elevated GFAP/NfL in patients with depression or positive associations with depression severity. Although limited by a small control group, our study may support the presence of glial dysfunction but not damage to neurons in depression.

1. Introduction

Major depressive disorder (MDD) affects an estimated 264 million people and is one of the largest disease burdens worldwide (GDB 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). Knowledge of the exact mechanisms behind MDD remains elusive, but neuroinflammation, along with brain glial dysfunction and neuronal atrophy, has received increasing attention as potential contributing factors (Kim et al., 2018; Orlovska-Waast et al., 2019; Rajkowska and Stockmeier, 2013). In line with this, postmortem and animal studies have indicated decreased numbers, density, and protein expression of glial cells and pyramidal neurons in the hippocampus and cortico-limbic

Abbreviations: BDI, Beck Depression Inventory; BMI, body mass index; hs-CRP, high-sensitive C-reactive protein; CSF, cerebrospinal fluid; GFAP, Glial Fibrillary Acidic Protein; HAM-D6, Hamilton Depression Rating Scale 6; HAM-D17, Hamilton Depression Rating Scale 17; IL6, interleukin 6; LLoD, lower limit of detection; LLoQ, lower limit of quantification; MDD, major depressive disorder; NfL, neurofilament light chain.

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brain regions of individuals with depression and animals with depressive-like symptoms (Kim et al., 2018; Paradise et al., 2012; Rajkowska and Miguel-Hidalgo, 2007; Rajkowska and Stockmeier, 2013; Reinés et al., 2004). This could suggest brain glial dysfunction and neuronal atrophy as a potential biological substrate, at least for subgroups of individuals with MDD.

The neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) are structural type III intermediate filaments that are key elements of the neuron and glia cell cytoskeleton (Abdelhak et al., 2022; Khalil et al., 2018). These proteins have been intensively investigated and demonstrated solid biomarker capabilities in a diverse range of neurological diseases of neurodegenerative, inflammatory, traumatic, and vascular origin (Abdelhak et al., 2022; Khalil et al., 2018). These investigations have soared with the development of ultra-sensitive methods that allow their detection in peripheral blood. However, evidence for the regulation of NfL and GFAP in depression is scarce (Mousten et al., 2022). Studies on serum and cerebrospinal fluid (CSF) NfL levels in uniform depression are small and with conflicting results (Al Shweiki et al., 2019; Besse et al., 2020; Gudmundsson et al., 2010; Mousten et al., 2022; Zachrisson et al., 2000). Likewise, initial studies on GFAP levels in MDD were conflicting, finding no changes (Gudmundsson et al., 2010; Xiong et al., 2014) or increased levels (Michel et al., 2021) in serum and CSF. Interestingly, Steinacker et al. (2021) recently presented compelling evidence for GFAP upregulation in serum from patients with depression. In a cohort of 45 patients with depression, serum GFAP levels were associated with depression severity and discriminated MDD from healthy controls with an area under the ROC curve of 0.70 and a sensitivity of 85 % (Steinacker et al., 2021). However, the study did not include a validation sample and should be interpreted cautiously. Moreover, the positive studies are hampered by small sample size or retrospective design as well as the lack of assessment of longitudinal associations between changes in the biomarker levels and clinical disease development (Michel et al., 2021; Steinacker et al., 2021) as well as the potential influence of psychotropic medication (Marathe et al., 2018).

This study compares serum NfL and GFAP levels in treatment-naïve patients with mild to moderate depression with NfL and GFAP levels in healthy controls. We investigate the association between depression severity, cognitive functioning, inflammatory biomarkers, and somatic parameters with serum NfL/GFAP. Moreover, we investigate the association between longitudinal changes in depression disease development and serum NfL/GFAP levels.

2. Materials and methods

2.1. Study cohort

The present study was conducted on biobank material from the DEMO-II trial (Krogh et al., 2012). The DEMO-II trial was a single-center RCT investigating the effect of three months of aerobic exercise versus stretching in patients with major depression. The DEMO-II included 56 patients in the aerobic training arm, 59 in the stretch control arm, and 57 healthy controls. Detailed information on the cohort has been published previously (Krogh et al., 2012). In brief, eligible patients were men and women (18–60 years) diagnosed with depression according to DSM-IV and assessed with the Danish version of the Mini International Neuropsychiatric Interview (MINI). Exclusion criteria included any antidepressant medication in the previous two months, current drug abuse, suicidal behavior, and current or previous psychotic or manic symptoms. The MINI screened the healthy controls to ensure they did not have current or previous psychiatric disease and were matched on sex, age, and body mass index (BMI).

The primary endpoint of the DEMO-II trial was change in the Hamilton Depression Rating Scale 17 (HAM-D17) score, and the secondary endpoints were measures of cognitive function and cardiovascular risk factors. Patients were followed-up after three months of training, but the trial demonstrated no effects on these endpoints. Therefore, patients with depression were pooled for the analysis performed in this investigation.

The trial was approved by the local ethical committee (H-A-2008-046) and the Danish Data Protection Agency (J.Nr. 2008-41-046) and is registered at ClinicalTrials.gov (NCT00695552).

2.2. Evaluation of mood and cognitive function

The severity of depression was assessed by the HAM-D17 as well as the 6-item scale (HAM-D6) (Hamilton, 1960), while the self-evaluated mood was quantified by Beck Depression Inventory (BDI)-II (Beck et al., 1961). Cognitive function was evaluated for six domains: verbal intelligence was examined by the Danish Adult Reading Test (Crawford et al., 1987), memory by Buschkes Selective Reminding Test (Buschke and Fuld, 1974), and Rey's complex figure test (Meyers et al., 1996), attention by the Digit Span Test (D, 1981), Serial Sevens (Smith, 1967), and Stroops Test (Alvarez and Emory, 2006), psychomotor speed by Digit Symbol Test (D, 1981) and Trail making A and B (Reitan, 1955), language by the Verbal Fluency Test-S and -Animals (Borkowski et al., 1967), and executive functions by the Design Fluency test (Baldo et al., 2001).

2.3. Physical examination

The physical examinations were performed on fasting subjects between 8 and 10 a.m. Height and weight were measured with an electronic weight, and waist circumference was measured twice and reported as the mean of these two measurements. BMI was calculated. Blood pressure was measured three times after 5 min of rest in a sitting position with a certified monitor reporting the mean. The VO_2 max was measured by a cardiopulmonary exercise test based on L.B. Andersen's protocol (Andersen, 1995).

Blood was collected from an indwelling catheter in the antecubital vein into EDTA anticoagulated tubes after 5 mins of rest in a sitting position. Samples were centrifuged and stored at −80 °C until further analysis.

2.4. Laboratory methods

Serum levels of NfL and GFAP were analyzed with the novel high sensitivity Simoa technology on an HD-I analyzer using the NfL advantage kit and the GFAP Discovery assay according to the manufacturer's instructions (Quanterix, Lexington, MA, USA). The NfL kit is validated for routine clinical analysis in our ISO15189 accredited laboratory and is under external quality control through the Alzheimer's Association quality control program for CSF biomarkers (Hviid et al., 2020). In this study, internal quality controls at two levels were used, and the intermediate precision at mean levels of 4.7 and 40.6 pg/ml were 8.6 % and 6.7 %. According to the manufacturer, the lower limit of detection (LLoD) is 0.038 pg/ml. The GFAP measurement is performed under internal quality control with in-house made controls at mean levels 123.4 pg/ml and 1692.5 pg/ml, which has an intermediate precision of 17.0 % and 22.1 %. According to the manufacturer, the calibration range is 0 to 1000 pg/ml; the LLoD is 0.211 pg/ml, and the lower limit of quantification (LLOQ) = 0.686 pg/ml. All analysis was performed as a batch in singles by a certified laboratory technician with special competencies in high-sensitivity analysis who were blinded to the status of the patients. Samples were analyzed at 4 times dilution. Routine laboratory procedure states that samples with levels below LLoD are to be at 4-times dilution, and samples above the calibration range are to be repeated at 10- or 100-times dilution. In this study, all samples were within the accepted range of measurement. Results for IL6 and high-sensitivity (hs) CRP has been published previously (Krogh et al., 2014). This study used the published data to explore the association between NfL and GFAP levels.

2.5. Statistics

A pre-study power calculation revealed 90 % power to detect a 33 % change in baseline levels of circulating NfL/GFAP with an alpha level of 0.05. Data distribution was assessed through inverse QQ plots. Baseline NfL and GFAP levels were compared by unpaired *t*-tests after ln-transformation. Group-wise comparisons of NfL and GFAP were adjusted for age, sex, and BMI using multiple regression. NfL or GFAP was analyzed as the dependent variable, with patient/control, age, sex, and BMI as independent variables. NfL and GFAP had a screwed distribution and were ln-transformed before the analysis. The regression coefficients and confidence intervals were transformed back to the original scale and are presented as percentage change per unit of the independent variable. The association of depression scores and cognitive items with NfL/GFAP at baseline was analyzed by multiple linear regression analysis. In the regression analysis, NfL or GFAP was expressed as the dependent variable, while depression and cognitive items were the independent variables. Change in GFAP/NfL, depression, and cognition scores from baseline to follow-up was expressed as delta values (Δ: follow-up—baseline) and analyzed by a one-sample t-test. The association of changes in NfL and GFAP with depression and cognition during follow-up was analyzed by a Spearman correlation of the delta values. Significant findings were further explored in a multiple linear regression model with ΔGFAP/ΔNfL as the dependent variable and Δdepression or Δcognitive score as the independent variable. The identification of confounders for serum NfL and GFAP levels was done through forward stepwise regression analysis with NfL/GFAP as the dependent variable and the somatic parameters as independent variables. Subsequent regression analyses were adjusted for identified confounders (BMI) as well as preplanned parameters (age and sex). The correlation between GFAP and NfL was assessed by Spearman's Rho and linear regression analysis, with NfL as the dependent variable and GFAP and depression as independent variables. The association between GFAP/NfL with IL6 and hs-CRP was analyzed in a regression model with GFAP/NfL as the dependent variable and IL6/hs-CRP as the independent variable and adjusted for identified confounders. All statistical analysis was performed in STATA version 15.5.5, and a *p*-value ≤ 0.05 was considered statistically significant.

2.5.1. Deviation from the analysis plan

The adjusted group-wise comparison of NfL/GFAP was changed from logistic regression to multiple linear regression after consultation with a statistician. Educational level was included in the confounder analysis after the reviewer's advice.

3. Results

3.1. Study cohort

Baseline blood samples from 110 patients with depression and 33 matched healthy controls were available for this study. Follow-up blood samples were obtained from 80 of the patients with depression. The baseline characteristics are displayed in Table 1. The groups were comparable with respect to sex, age, and all selected somatic parameters except VO_2 max (Table 1).

Table 1
Baseline characteristics of included patients with depression and healthy controls.

	Patients, n = 110	Controls, n = 33	p-Value
Age, years, mean (SD)	42 (11)	39 (14)	0.21
Sex (m/f), n (%)	38/72 (35/65 %)	10/23 (30/70 %)	0.65
High school education, n (%)	54 (49)	26 (79)	**<0.01**
Weight, kg, mean (SD)	77.8 (20.4)	76.5 (20.1)	0.74
Height, cm, mean (SD)	170.9 (9.4)	172.3 (9.5)	0.47
BMI, kg/m^2, mean (SD)	26.4 (6.0)	25.9 (7.5)	0.68
Waist circumference, cm, mean (SD)	89.8 (16.6)	84.8 (12.9)	0.12
Systolic BP, mmHg, mean (SD)	122.9 (16.6)	119.1 (12.9)	0.23
Diastolic BP, mmHg, mean (SD)	82.3 (11.3)	79.6 (10.1)	0.22
V02 max, ml/kg/min, mean (SD)	25.4 (7.1)	29.9 (6.9)	**<0.01**
Depression			
HAM-D17, mean (SD)	18.9 (3.9)	0.6 (0.8)	**<0.001**
0–6, n (%)	0	33 (100)	
7–12, n (%)	3 (3)		
13–17, n (%)	36 (33)		
18–24, n (%)	62 (56)		
>24, n (%)	9 (8)		
HAM-D6	10.4 (0.48)	0.2 (0.1)	**<0.001**
BDI	35.4 (7.6)	1.4 (1.7)	**<0.001**
Currently on sick leave, n (%)	37 (34)	0 (0)	**<0.001**
Recurrent depression, n (%)	54 (49)		
>3 previous episodes of depression, n (%)	8 (7)		
Prior suicidal attempt, n (%)	7 (6)		
Cognitive function, mean (sd)			
Intelligence			
DART	34.2 (8.8)	35.3 (9.0)	0.54
Memory			
Reys complex figure test	21.1 (6.3)	23.4 (4.7)	**0.05**
Digit span test, forward	6.4 (2.1)	6.5 (1.9)	0.82
Buschke, total	17.0 (11.7)	13.1 (9.5)	0.08
Attention			
Digit span test, backward	5.3 (1.9)	5.5 (2.1)	0.51
Subtracting serial seven	7.9 (2.8)	7.7 (2.8)	0.78
Stroop test, crosses	78.1 (21.1)	66.6 (12.7)	**<0.01**
Stroop test, congruent	60.4 (16.3)	52.2 (7.9)	**<0.01**
Stroop test, incongruent	126.8 (36.9)	106.4 (28.6)	**<0.01**
Psychomotor speed			
Trail making A	33.6 (13.8)	24.4 (8.2)	**<0.001**
Trail making B	78.4 (29.9)	58.0 (15.1)	**<0.001**
Digit symbol test	45.8 (9.6)	51.9 (9.3)	**<0.01**
Language			
Verbal fluency S	12.1 (4.8)	13.9 (5.8)	0.07
Verbal fluency animals	20.8 (6.0)	24.8 (5.9)	**<0.001**
Executive function			
Design fluency	28.6 (6.4)	31.3 (5.8)	**0.03**

Statistically significant findings displayed in bold

3.2. Association between somatic baseline variables with NfL and GFAP

The association of NfL and GFAP with the somatic parameters age, sex, alcohol consumption, BMI, Vo2 max, systolic- and diastolic blood pressure, as well as educational level, were explored in a stepwise regression model including data from both patients and controls (Supplement Table 1). This identified a negative association between BMI with NfL [−1.5 % (95 % CI: −2.4 to −0.5), $p < 0.01$] and GFAP [−1.5 % (95 % CI: −2.6 to −0.3), $p = 0.01$] and confirmed a positive association with age for [2.7 % (95 % CI: 2.2 to 3.2), $p < 0.001$] and GFAP [1.5 % (95 % CI: 0.8 to 2.1), $p < 0.001$]. Sex was not significantly associated with NfL/GFAP but was included in subsequent analysis according to the analysis plan.

3.3. Serum NfL and GFAP in patients with depression vs. healthy controls

Serum levels of NfL and GFAP were above the LLOQ in all samples. Median NfL and GFAP levels in patients with depression and healthy controls are displayed in Fig. 1. Baseline levels of NfL and GFAP in patients with depression were comparable to levels observed in healthy controls in the crude analysis ($p > 0.20$ for both). However, after adjustment for age, sex, and BMI, baseline serum GFAP levels were significantly lower among patients with depression than healthy controls [−16.8 % (95 % CI: −28.8 to −1.9), $p = 0.03$]. By contrast, NfL baseline levels in patients with depression remained comparable with healthy controls after this adjustment [−3.6 % (−16.1 to 9.0), $p = 0.57$].

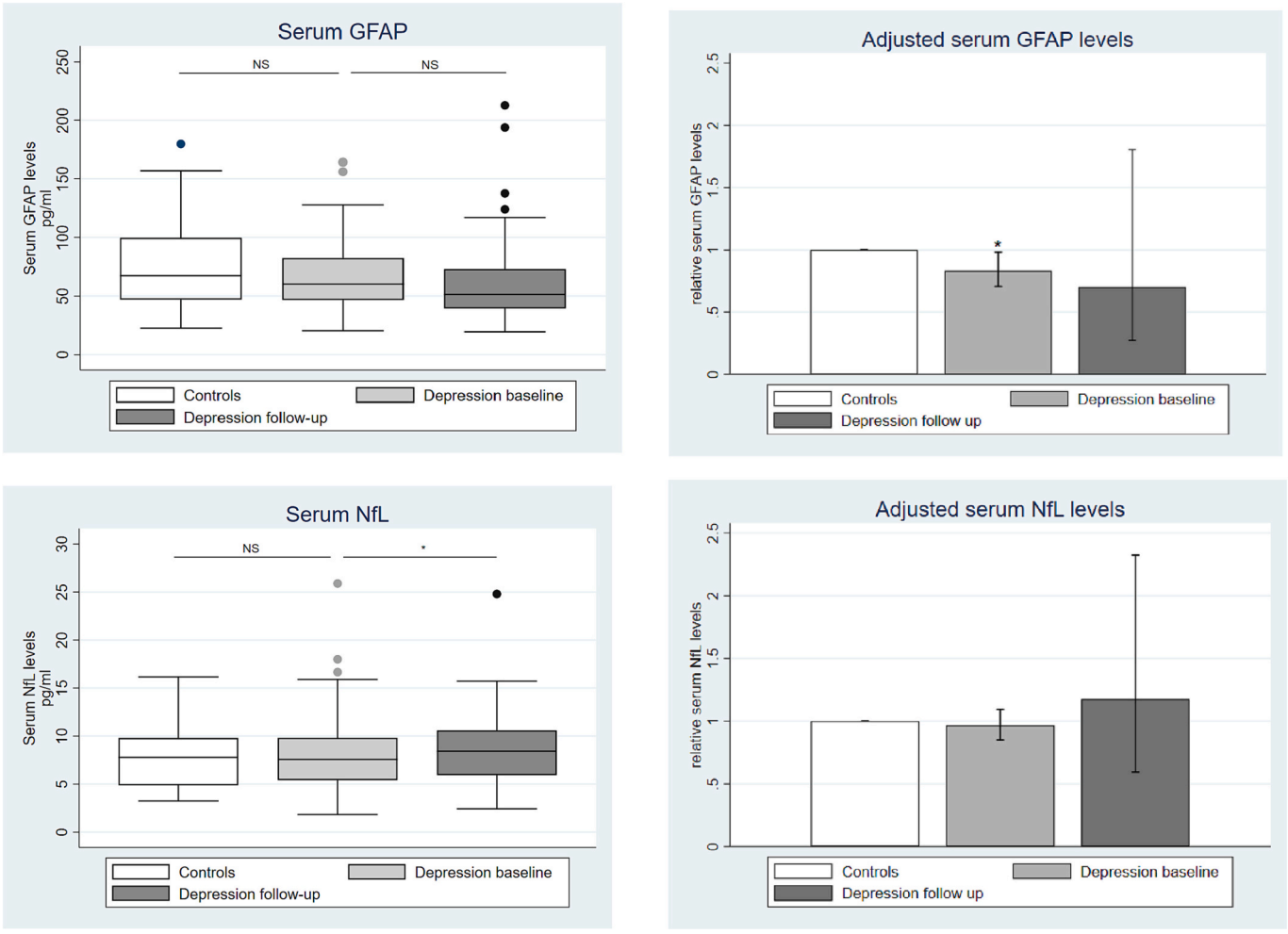

Fig. 1. Serum GFAP and NfL levels in patients with depression and healthy controls.
Boxplot of unadjusted serum levels of GFAP and NfL (A) and adjusted levels of GFAP and NfL (B). Level in patients with depression at baseline ($n = 110$) and at three-month follow-up ($n = 80$) as well as in mentally healthy controls ($n = 33$) displayed. Bar chart of relative serum levels of NfL (c) and GFAP (d) after adjustment for age, sex, and body mass index. Boxes indicate median with interquartile range. Whiskers are adjacent lines, and dots are extreme values. Bars indicate relative mean levels with standard deviation. *$p < 0.05$.

Among patients with depression, longitudinal measurements of NfL levels increased from baseline to follow-up (μ = 0.68 pg/ml, p = 0.03), while the longitudinal GFAP levels did not differ significantly (μ = −3.58 pg/ml, $p = 0.24$).

3.4. Baseline depression and cognition scores' association with NfL/GFAP in patients with depression

As displayed in Table 2, no significant associations between GFAP and NfL with HAM-D17 score or BDI were observed among patients with depression. In the crude analysis, HAM-D6 was not associated with NfL, but after adjustment for BMI, age, and sex, HAM-D6 and NfL levels were positively associated [2.8 % (95 % CI: 0.0 to 5.7 %), $p = 0.05$]. The cognitive tests DART and Reys recall test were positively associated with baseline NfL levels, but the association disappeared in the adjusted analysis. Stroops crosses test was positively associated with baseline serum NfL levels. The associations were further explored in a sensitivity analysis comparing the associations in patients with mild (HAM-D17 < 18) vs. moderate-to-severe depression (HAM-D17 ≥ 18) (supplement Table 2). This analysis reproduced the association between NfL and Stroops crosses in the moderate-to-severe group, whereas the association between NfL and HAM-D6 was seen only among patients with mild depression (supplemental Table 2). We observed no significant associations between GFAP with depression or cognition item scores in the unadjusted, adjusted, or sensitivity analysis.

3.5. Longitudinal changes in depression, cognition scores, and NfL/GFAP in patients with depression

The associations between NfL/GFAP change with improvement in depression and cognitive items are presented in Table 3. Depression scores improved during follow-up [Mean differences: HAM-D17: μ = −7.9 (SD 6.8), $p < 0.001$; HAM-D6; μ = −4.3 (SD 4.2), p < 0.001; BDI-II: -14.0 (SD 11.6), p < 0.001]. The associations of the Reys test with GFAP and Trail making A with NfL were further explored in a multiple linear regression model adjusted for age, sex, and BMI. In the univariate analysis, trail making A and NfL levels were negatively associated [−5.5 % (95 % CI: −9.8 to −1.1), $p = 0.01$], and this association was unaffected by adjustment for age, sex, and BMI [−5.5 % (−9.9 to −1.0), $p = 0.02$]. There was no statistically significant association between GFAP and Reys test in the univariate or adjusted analysis ($p = 0.07$). The potential correlation between a change in BMI from baseline to follow-up with a change in NfL or GFAP was also explored but was not statistically significant [NfL (rho = −0.029, $p = 0.82$); GFAP (rho = −0.053, $p = 0.69$)].

Table 2
Association (β) between depression severity and cognitive function with NfL and GFAP in patients with depression at study inclusion.

	NfL inclusion levels, crude	NfL inclusion levels, adjusted[a]	GFAP inclusion levels, crude	GFAP inclusion levels, adjusted[a]
Depression				
HAM-D17	0.8 % (−1.5 to 3.1) $p = 0.51$	0.1 % (−1.6 to 1.8) $p = 0.90$	1.3 % (−0.9 to 3.5) $p = 0.26$	0.6 % (−1.5 to 2.8) $p = 0.55$
HAM-D6	2.0 % (1.9 to 6.0) $p = 0.31$	**2.8 % (0.0 to 5.7) p = 0.05**	0.6 % (−3.0 to 4.4) $p = 0.74$	0.8 % (−2.7 to 4.4) $p = 0.67$
BDI-II	−0.8 % (−1.9 to 0.4) $p = 0.20$	0.1 % (−0.8 to 0.9) $p = 0.92$	0.3 % (−0.8 to 1.3) $p = 0.61$	0.5 % (−0.5 to 1.6) $p = 0.30$
Recurrent depressive episodes	4.7 % (−23.6 to 43.5) $p = 0.77$	2.1 % (−18.4 to 27.8) $p = 0.86$	3.8 % (−21.6 to 37.5) $p = 0.79$	3.4 % (−20.5 to 34.5) $p = 0.80$
Cognitive function				
Intelligence				
DART	**1.8 % (0.7 to 2.9) $p = 0.001$**	0.5 % (−0.3 to 1.4) p = 0.24	0.7 % (−0.3 to 1.7) $p = 0.18$	0.1 % (−1.0 to 1.2) p = 0.82
Memory				
Reys complex figure test	**−2.2 % (−3.6 to −0.8) p < 0.01**	−1.0 % (−2.0 to 0.1) $p = 0.09$	−0.01 % (−1.4 to 1.4) $p = 0.99$	0.5 % (−0.9 to 1.9) $p = 0.46$
Digit span test, forward	1.7 % (−2.6 to 6.3) $p = 0.44$	0.1 % (−3.0 to 3.4) $p = 0.94$	0.7 % (−3.3 to 4.6) p = 0.74	−0.4 % (−4.2 to 3.4) $p = 0.82$
Buschke, total	0.5 % (−0.3 to 1.3) p = 0.18	0.15 % (−0.43 to 0.7) p = 0.61	0.2 % (−0.6 to 1.0) $p = 0.62$	0.04 % (−0.7 to 0.8) $p = 0.91$
Attention				
Digit span test, backward	−0.2 % (−4.9 to 4.6) $p = 0.95$	−2.0 (−5.5 to 1.4) p = 0.24	−2.2 % (−6.5 to 2.1) $p = 0.32$	−3.0 % (−7.0 to 1.1) $p = 0.16$
Subtracting serial seven	−2.3 % (−6.1 to 1.6) p = 0.25	−0.5 % (−5.3 to 0.4) $p = 0.09$	2.3 % (−1.1 to 5.8) p = 0.17	2.4 % (−0.9 to 5.6) $p = 0.15$
Stroops test, crosses	**0.5 % (0.07 to 0.9) p = 0.02**	**0.3 % (0.03 to 0.6) p = 0.03**	0.3 % (−0.1 to 0.7) $p = 0.11$	0.3 % (−0.1 to 1.0) $p = 0.17$
Stroop test, congruent	0.4 % (−0.2 to 0.9) $p = 0.21$	0.3 % (−0.1 to 0.7) p = 0.16	0.3 % (−0.2 to 0.8) p = 0.25	0.3 % (−0.2 to 0.8) $p = 0.25$
Stroop test, incongruent	0.0 % (−0.2 to 0.3) p = 0.79	0.1 % (−0.08 to 0.3) $p = 0.28$	0.0 % (−0.2 to 0.3) $p = 0.71$	0.1 % (−0.1 to 0.3) $p = 0.37$
Psychomotor speed				
Trail making A	0.1 % (−0.6 to 0.8) p = 0.77	−0.1 (−0.6 to 0.4) p = 0.68	0.04 (−0.7 to 0.7) p = 0.91	0.2 % (−0.7 to 0.7) $p = 0.96$
Trail making B	0.1 % (−0.3 to 0.4) $p = 0.65$	0.03 % (−0.2 to 0.3) p = 0.77	−0.04 % (−0.3 to 0.3) p = 0.82	0.05 % (−0.3 to 0.2) p = 0.74
Digit symbol test	−0.8 % (−1.8 to 0.2) $p = 0.10$	−0.4 % (−1.0 to 0.3) p = 0.32	−0.06 % (−1.0 to 0.9) $p = 0.89$	0.2 % (−0.7 to 1.1) $p = 0.68$
Language				
Verbal fluency S	0.6 % (−1.2 to 2.5) $p = 0.50$	−0.4 % (−1.7 to 1.0) $p = 0.56$	0.3 % (−1.5 to 2.0) p = 0.77	−0.2 % (−1.9 to 1.5) $p = 0.84$
Verbal fluency animals	1.0 % (−0.6 to 2.5 %) p = 0.25	−0.1 % (−1.3 to 1.0) p = 0.80	0.7 % (−0.8 to 2.1) $p = 0.35$	0.2 % (−1.2 to 1.6) $p = 0.78$
Executive function				
Design fluency	0.3 % (−1.1 to 1.7) $p = 0.64$	−0.3 (−1.4 to 0.6) p = 0.46	0.6 % (−0.7 to 1.9) $p = 0.34$	0.2 % (−1.1 to 1.4) p = 0.79

Results of the crude association and a multiple linear regression analysis adjusted for age, sex, and body mass index are shown. In each analysis, Ln-transformed NfL and GFAP were analyzed as the dependent variable. The regression coefficients with their 95 % CI were transformed back to the original scale and are expressed as percentage change per unit of each independent factor.
Abbreviations: Hamilton Depression Rating Scale 17/6 (HAM-D17/D6); Beck Depression Inventory (BDI)-II; Danish Adult Reading Test (DART).
Statistically significant findings displayed in bold
[a] Adjusted for age, sex, and body mass index.

Table 3
Spearman correlation between longitudinal changes in NfL and GFAP with change in depressive and cognitive symptoms.

	ΔNfL		ΔGFAP	
	Spearman's rho	p-Value	Spearman's rho	p-Value
ΔDepression				
HAM-D17	0.04	0.75	0.18	0.16
HAM-D6	0.12	0.32	0.22	0.09
BDI-I	0.17	0.21	0.14	0.32
ΔCognitive function				
Memory				
Reys complex figure test	−0.14	0.29	**−0.29**	**0.03**
Digit span test, forward	−0.03	0.79	−0.09	0.51
Buschke, total	−0.05	0.70	0.06	0.65
Attention				
Digit span test, backward	0.09	0.49	0.06	0.63
Subtracting serial seven	−0.03	0.81	0.25	0.09
Stroop test, crosses	0.05	0.70	−0.10	0.46
Stroop test, congruent	−0.05	0.72	−0.04	0.80
Stroop test, incongruent	0.17	0.20	0.14	0.32
Psychomotor speed				
Trail making A	**−0.25**	**0.04**	−0.08	0.54
Trail making B	0.15	0.25	0.24	0.07
Digit symbol test	−0.04	0.75	−0.01	0.92
Language				
Verbal fluency S	0.13	0.31	−0.01	0.96
Verbal fluency animals	−0.03	0.81	−0.18	0.17
Executive function				
Design fluency	0.18	0.15	−0.03	0.83

Statistically significant findings displayed in bold

3.6. Association between NfL, GFAP, and inflammation

Pooled baseline levels of NfL and GFAP from patients and controls were significantly correlated [$\beta = 0.62$ (95 % CI 0.26 to 0.97), $R^2 = 0.11$], but no significant effect of, or interaction with, depression was observed (both, $p > 0.09$). The potential association between pooled baseline NfL and GFAP levels with serum interleukin-6 and hs-CRP was explored in a regression model but did not reach statistical significance or reveal a significant effect of depression ($p > 0.21$ for all).

4. Discussion

The present study is the largest investigation of serum GFAP and NfL levels in treatment-naïve patients with depression, also exploring the association between GFAP/NfL levels with somatic parameters and inflammatory biomarkers. GFAP levels were significantly lower at baseline in patients with depression than in healthy controls after adjustments. However, GFAP levels were not consistently associated with depression severity or cognitive scores at baseline or with longitudinal changes in mood or cognition during follow-up. In patients with depression, mean NfL levels increased from baseline to follow-up, while GFAP levels were unchanged. We did not observe consistent associations of NfL with depression or cognition or between biomarkers of inflammation with serum GFAP/NfL in this study.

Our observation of reduced GFAP levels among patients with depression should be interpreted carefully as associations with disease severity and development were absent, and the associations were present only in the adjusted analyses. Prior reports on GFAP levels in depression are conflicting and hampered by small sample sizes or methodological issues (Gudmundsson et al., 2010; Michel et al., 2021; Steinacker et al., 2021; Xiong et al., 2014). In a small study of 11 patients with depression, Gudmundsson et al. (2010) reported comparable GFAP levels in patients compared with controls. In the largest study available, GFAP levels were assayed in 240 patients with depression or bipolar disorder and 260 controls, but all measurements were below the LLOQ of the assay used (Xiong et al., 2014), whereas all measurements were above LLOQ in our analyses. Recently, two studies reported significantly increased GFAP levels in the serum of 45 patients with moderate to severe depression (Steinacker et al., 2021) and in CSF (Michel et al., 2021) of 102 patients with severe depression. However, the controls groups in the studies were small, ranging from 16 to 39 (Michel et al., 2021; Steinacker et al., 2021) and, in the study by Michel et al. (2021), the 39 persons in the control group suffered from intracranial hypertension. By contrast, postmortem studies of patients with depression have mainly found decreased tissue expression of GFAP (Cobb et al., 2016; Fatemi et al., 2004; Si et al., 2004; Webster et al., 2005), which is in line with our findings. While the exact cause for these conflicting results remains unclear, psychotropic medication may affect measurable levels of neurological biomarkers in the CSF (Jakobsson et al., 2014) and potentially also in serum. Indeed, postmortem tissue studies have reported higher GFAP expression in biopsies from depressed patients receiving medical treatment compared with those not treated with psychotropic medication (Cobb et al., 2016). None of the patients in our study received antidepressants within the last two months before recruitment, while >97 % of patients in the positive studies received antidepressant treatment (Michel et al., 2021; Steinacker et al., 2021). Along this line, less than one in four of the patients in the previous negative study by Gudmundsson et al. (2010) were on psychotropic medication. This may indicate that medical treatment rather than the disease could cause the previously observed GFAP elevations in patients with depression. Furthermore, the previous studies did not correct for patient BMI, and as we reproduced a previously reported negative association between the biomarkers and BMI (Rebelos et al., 2022), not accounting for BMI might also have affected the findings of the previous studies.

NfL is considered a biomarker of neuroaxonal damage, and as such, it is comprehendible that serum NfL levels were comparable between patients and controls in our study (Khalil et al., 2018), and our results are generally in line with the current literature (Al Shweiki et al., 2019; Besse et al., 2020; Steinacker et al., 2021). Still, Gudmundsson et al. (2010) reported elevated NfL levels in the CSF of geriatric patients with depression; however, it may be speculated that a component of neurodegeneration is involved when studying depression in an elderly population (Fiske et al., 2009). This could explain the increased levels of NfL observed in geriatric patients with depression, which is not evident in studies on younger patients. Further studies are required to explore this possibility. Nonetheless, we observed a positive association of NfL with the HAM-D6 score in patients with depression. HAM-D6 has been suggested to capture depression better than other scales (Timmerby et al., 2017). However, the association was relatively weak, not reflected in other tested measures of depression severity, and did not follow changes in depression scores during follow-up. In the sensitivity analysis, we reproduced the association between NfL and HAM-D6 among patients with mild depression, and associations with cognition items in patients with mild vs. moderate to severe depression were inconsistent. This warrants a cautious interpretation as the association between NfL and HAM-D6 could result from a statistical type-I error.

Our detailed investigation of the association of GFAP and NfL with cognitive items did not support a consistent association. This is at odds with studies on older patients with mild cognitive impairment, where the cognitive impairment in patients with Alzheimer's or diabetes was shown to be associated with GFAP and NfL levels (Ayala-Guerrero et al., 2022; Mattsson et al., 2017). To the best of our knowledge, the association of GFAP/NfL with cognitive functioning has not previously been tested in unipolar depression. As such, our data suggest that the underlying mechanisms of affected cognitive functioning in mild to moderate unipolar depression might be distinct from the underlying mechanisms of the affected cognition observed in Alzheimer's or diabetes.

The limitations of our study include that it is conducted on biobank material from a trial not specifically designed for the present study. Still, the design is sufficient for the tests performed in our study and for the longitudinal monitoring of biomarkers. We pooled samples from patients subjected to different exercise interventions, which could potentially affect the results. However, previous studies have not revealed a difference in depression or cognitive performance as well as investigated biochemical biomarkers between the intervention groups (Krogh et al., 2014; Krogh et al., 2012). It is therefore considered unlikely to have affected the results of our study. Our patients with depression may have been less severely ill based on the depression scores than the populations investigated where effects on NfL/GFAP were demonstrated (Michel et al., 2021; Steinacker et al., 2021). Nevertheless, a dose-response relationship has previously been observed (Steinacker et al., 2021), suggesting that at least some effect should have been present also in our cohort. Only serum samples were available for our studies which invariably has limitations compared with CSF analysis. However, studies across various patient groups and healthy controls have found a strong correlation between serum and CSF levels of the neurobiomarkers investigated in this study (Abdelhak et al., 2022; Khalil et al., 2018). We did not adjust our analysis for multiple testing, which may have resulted in type-I errors, and also, the number of healthy controls in our cohort was small. Thus, we interpreted our results critically in that context. Lastly, the GFAP assay had a relatively high intermediate precision, reducing the ability to detect small differences. It cannot be ruled out that this may have affected the observed results, but we alleviated the effect by analyzing samples in random batches.

In summary, we found evidence of reduced serum GFAP and unaffected NfL in our cohort of treatment-naïve patients with unipolar depression compared to healthy controls. As such, our study supports glial dysfunction and opposes damage to neurons in this patient group. Our data may indicate that previously observed GFAP or NfL changes in patients with depression could be due to treatment with psychotropic

medication and confounding effects of BMI and age, and further studies are needed to clarify these aspects.

CRediT authorship contribution statement

CVBH, MEB, and SHC conceived and designed the study, JK and MN acquired the data, and CVBH, MEB, and JK analyzed and interpreted the data. CVBH drafted the paper while the other authors revised it critically. The present manuscript has been read and approved in its present form by all authors who agree to be accountable for all aspects of the published work.

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Declaration of competing interest

CVBH: None; MEB: None; JK: None; MN: None; SCH: none.

Data availability

Data will be made available upon reasonable request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jad.2023.06.028.

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