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Increased serum glial fibrillary acidic protein associates with microstructural white matter damage in multiple sclerosis GFAP and DTI

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ABSTRACT

Background: Astrocytes and microglial cells are now recognized as active players in contributing to the diffuse neuroaxonal damage associated with disease progression of multiple sclerosis (MS). The serum level of glial fibrillary acidic protein (GFAP), a biomarker for astrocytic activation, is increased in MS and associates with disease progression and disability. Similarly, diffusion tensor imaging (DTI) parameters for microstructural changes in brain, including demyelination and axonal loss, associate with disability. The association between brain DTI parameters and serum GFAP has not been previously explored in MS. The objective of the study was to get insights into DTI-measurable pathological correlates of elevated serum GFAP in the normal appearing white matter (NAWM) of MS.

Methods: A total of 62 MS patients with median age of 49.2 years were included in the study. Study patients underwent DTI-MRI and blood sampling for GFAP determination by single molecule array (Simoa). Mean fractional anisotropy (FA) and mean (MD), axial (AD) and radial (RD) diffusivities were calculated within the entire NAWM and six segmented NAWM regions. The associations between the DTI parameters and GFAP levels were analysed using Spearman correlation analysis and multiple regression model with sex and disease modifying treatment (no, 1st line or 2nd line) as adjustments.

Results: Elevated serum GFAP levels correlated significantly with decreased FA values within the entire ($\rho = -0.39$, $p = 0.03$), frontal ($\rho = -0.42$, $p = 0.02$), temporal ($\rho = -0.37$; $p = 0.04$) and cingulate ($\rho = -0.38$, $p = 0.034$) NAWM, and increased MD and RD within the frontal NAWM ($\rho = 0.36$, $p = 0.046$ for both). Similarly, higher GFAP associated with lower FA in frontal and cingulate NAWM in the multiple regression model corrected for confounding variables (standardised regression coefficient $\beta = -0.29$, $p = 0.045$ and $\beta = -0.33$, $p = 0.025$).

Conclusions: Our results give evidence that increased serum GFAP levels associate with DTI-measurable microdamage in the NAWM in MS. Our work supports the use of serum GFAP as a biomarker for MS pathology-related astrocytopathy and related diffuse white matter damage.

Abbreviations: AD, Axial diffusivity; CNS, Central nervous system; DTI, Diffusion tensor imaging; EDSS, Expanded Disability Status Scale; FA, Fractional anisotropy; GFAP, Glial fibrillary acidic protein; MD, Mean diffusivity; MS, Multiple sclerosis; NAWM, Normal appearing white matter; NfL, Neurofilament light chain; PF, parenchymal fraction; RD, Radial diffusivity.

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1. Introduction

The complex pathogenesis of MS involves both adaptive and innate immune cells. Within the central nervous system (CNS), the glial compartment including astrocytes and microglial cells is now recognized as active player in contributing to the diffuse neuroaxonal damage associated with disease progression. Reactive astrocytes are present both in the demyelinating lesions and in the adjacent normal appearing white matter (NAWM) (Ponath et al., 2018). Glial fibrillary acidic protein (GFAP) is the main intermediate filament of astrocytes in humans (Petzold, 2015). The expression of GFAP is up-regulated in reactive astrogliosis and is considered as a biomarker for astrocytic activation. Brain injury leads to release of GFAP from injured astrocytes into interstitial/extracellular fluid, CSF and finally into blood circulation via the glymphatic pathway (Yang and Wang, 2015). The serum levels of both GFAP and neurofilament light chain (NfL), a biomarker for axonal damage, are increased in MS and associate with disease progression and disability (Abdelhak et al., 2018; Ayrignac et al., 2020; Barro et al., 2018; Cantó et al., 2019; Högel et al., 2020; Varhaug et al., 2019). Similarly, diffusion tensor imaging (DTI) findings reporting on microstructural changes in brain, including demyelination and axonal loss, associate with disability in MS (Andersen et al., 2018; Bezukladova et al., 2020). Brain DTI scalars correlate with serum NfL concentration (Saraste et al., 2021), but not much is known about the association between brain DTI-findings and serum GFAP concentration in MS. As astrocytes may be an important contributor to the neuroaxonal damage in MS brain, we wanted to explore in this work whether there is an association between serum GFAP potentially released from reactive astrocytes, and DTI-measurements of the diffuse NAWM damage within the brain.

2. Patients and Methods

2.1. Standard protocol approvals and patient consents

The study was approved by the Ethical Committee of the Hospital District of Southwest Finland. A written informed consent was obtained from all participants according to the Declaration of Helsinki.

2.2. Study patients and protocols

A total of 62 MS patients with either relapsing remitting (n=39) or secondary progressive (n=23) disease type, recruited from the Neurology outpatient clinic of the Division of Clinical Neurosciences at the Turku University Hospital, Turku, Finland, and 10 healthy controls were included in the study. Blood samples were collected, and serum was stored at -40℃ within 4 hours of sampling. Serum concentration of GFAP was measured in duplicates (mean coefficient of variation 3.4 %) using commercially available kit and single molecule array (Simoa) assay technology (Högel et al., 2020). The NfL levels were measured from the same samples for comparison. Brain MRI including DTI sequences was performed with 3T MRI Phillips Ingenuity (Philips Healthcare, Cleveland, OH) scanner in Turku PET Centre as previously described (Bezukladova et al., 2020). NAWM region of interest was created in the images, and the DTI data were pre-processed and analysed as described previously (Bezukladova et al., 2020). Briefly, to create the NAWM region of interest, T2 hyper-intense lesions were excluded from the entire white matter. Parenchymal fractions of volumetric MRI data were calculated by dividing volumes by intracranial volume. Mean values for fractional anisotropy (FA) and mean, radial and axial diffusivities (MD, RD and AD) were calculated both within the whole and segmented NAWM (frontal, parietal, temporal, occipital, cingulate and deep). MS patients were subdivided to GFAP(high) and GFAP(low) groups based on the median value of GFAP.

Table 1
Clinical characteristics of MS patients.

	all MS	GFAP(low)	GFAP(high)	p-value (low vs. high)
Number of patients	62	31	31	
Age	49.2 (43.7-54.5)	48.0 (41.5-51.7)	49.4 (44.9-59.4)	0.076[e]
Sex (female/male)	45/17	21/10	24/7	0.393[f]
Disease duration[a]	13.7 (10.1-20.0)	13.5 (9.8-14.4)	16.6 (10.7-25.9)	**0.017**[e]
Disease type (RRMS/SPMS)	39/23	23/8	16/15	0.066[f]
EDSS	3 (2-4)	2.5 (2-3.25)	3.5 (3-5)	**0.004**[e]
Treatment[b]				0.137[f]
no DMT	19	8	11	
1st line DMT[c]	17	12	5	
2nd line DMT[d]	26	11	15	
Biomarkers				
serum NfL (pg/ml)	22.52 (16.1-32.0)	18.99 (14.5-28.5)	29.43 (18.4-38.8)	**0.019**[e]
serum GFAP (pg/ml)	97.73 (68.7-129.2)	68.39 (61.5-84.5)	129.4 (109.8-173.9)	**<0.001**[e]
Volumes				
WM (cm^3)	454.5 (413-485)	423.4 (406-480)	467.1 (429-490)	0.097[e]
WM (PF)	0.33 (0.31-0.35)	0.33 (0.31-0.35)	0.33 (0.30-0.35)	0.97[e]
Cortical GM (cm^3)	428.2 (409-459)	424.1 (409.4-450.8)	431.3 (407-462)	0.46[e]
Cortical GM (PF)	0.32 (0.30-0.33)	0.32 (0.31-0.33)	0.31 (0.29-0.33)	0.25[e]
NAWM (cm^3)	440.7 (392-479)	418.0 (391-473.2)	451.7 (403-480)	0.33[e]
NAWM (PF)	0.32 (0.29-0.34)	0.32 (0.30-0.34)	0.32 (0.29-0.34)	0.6[e]
T1 lesion (cm^3)	4.1 (2.3-8.7)	3.4 (2.3-5.0)	6.5 (2.5-10.9)	0.094[e]
T2 lesion (cm^3)	11.5 (4.3-19.9)	6.7 (3.8-13.5)	15.1 (6.2-28.3)	**0.011**[e]

Median (interquartile range) values are shown expect for sex, disease type and treatment where number of patients are shown. MS patients were divided into GFAP(low) and GFAP(high) subgroups based on the median value of GFAP (97.73 pg/ml). Significant p-values are bolded. DMT = disease modifying treatment; EDSS = Expanded Disability Status Scale; GFAP = Glial fibrillary acidic protein; GM = grey matter; NAWM = normal appearing white matter; NfL = neurofilament light chain; MS = multiple sclerosis; PF = parenchymal fraction; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; WM = white matter.

[a] years from the onset of symptoms;
[b] at the time of investigations;
[c] interferon-beta, dimethyl fumarate, glatiramer acetate, teriflunomide;
[d] fingolimod, natalizumab, rituximab;
[e] Wilcoxon rank-sum test;
[f] Chi-square test

2.3. Statistical analysis

The statistical analysis was performed using R statistical software (version 4.0.0). Wilcoxon rank-sum and Chi-squared tests were used to compare differences between groups. The associations of GFAP with DTI parameters and parenchymal fractions of volumetric MRI data were analysed using Spearman correlation analysis and multiple regression model with sex and disease modifying treatment [no treatment, 1st line (dimethyl fumarate, glatiramer acetate, interferon-beta, teriflunomide) or 2nd line (fingolimod, natalizumab, rituximab)] as adjustments. In multiple regression model the logarithm of GFAP was used as the response to avoid the non-normality of residuals. In all analyses $p = 0.05$ was used as the threshold for statistical significance.

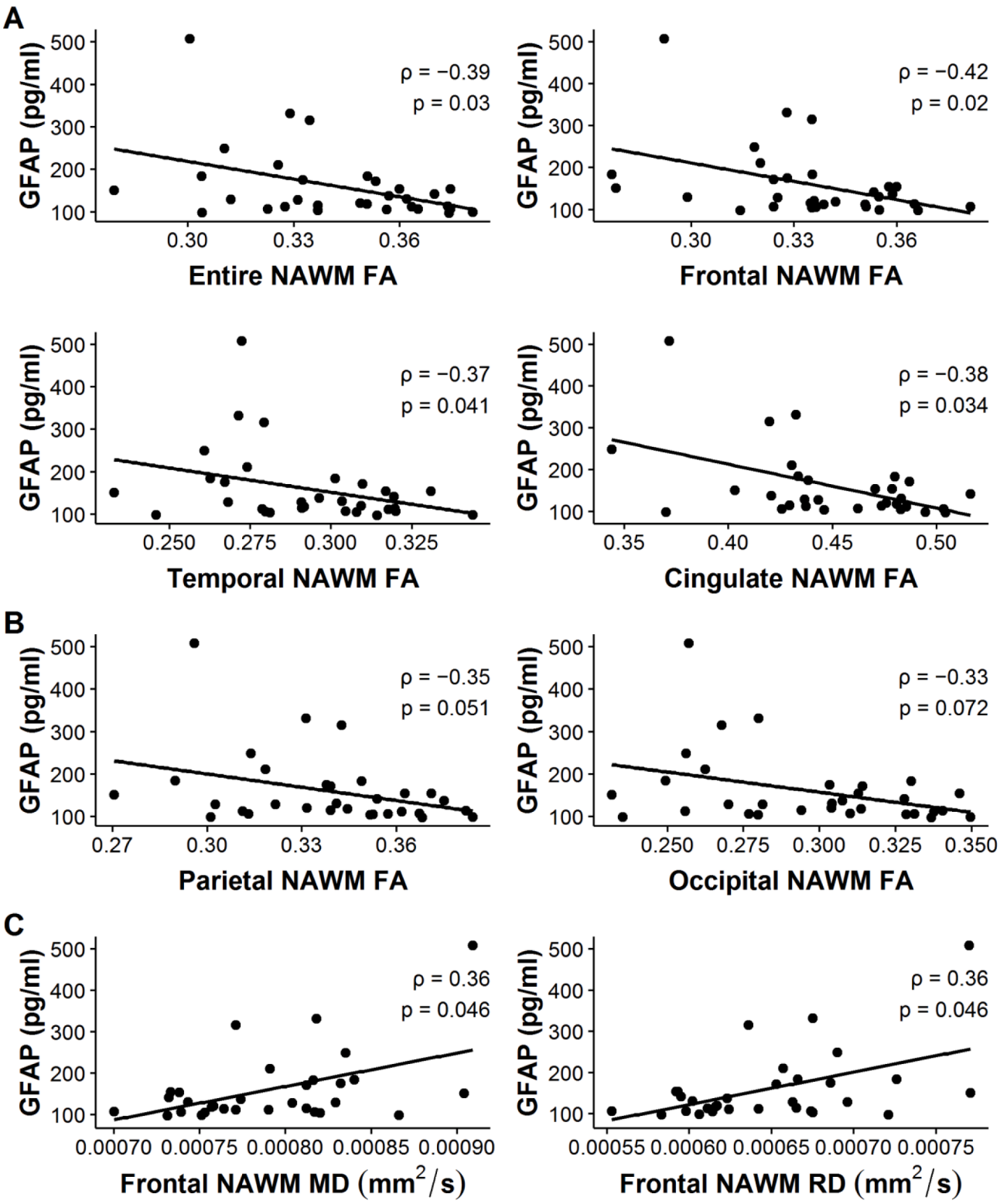

Fig. 1. Spearman correlations of serum GFAP with DTI indices of the entire and segmented NAWM. (A) Serum GFAP level correlated with fractional anisotropy (FA) in the entire, frontal, temporal and cingulate normal appearing white matter (NAWM). (B) In parietal and occipital NAWM the correlation between GFAP and FA did not reach statistical significance. (C) Serum GFAP correlated with the mean and radial diffusivities (MD and RD) in the frontal NAWM. Shown are Spearman correlation coefficients (ρ) and p-values among the patients of the GFAP(high) subgroup.

3. Results

The demographics and clinical characteristics of MS patients are presented in Table 1. Healthy controls did not differ from MS patients in age or sex (47.6 years, p = 0.7; 7 females, 3 males, p = 0.9). Serum GFAP was increased in MS patients compared to healthy controls (97.7 pg/ml vs. 67.5 pg/ml, p = 0.023), whereas there was no difference in the median serum NfL level between MS patients and healthy controls

Table 2
Statistically significant associations between serum GFAP concentrations and DTI-MRI indices.

	Spearman		Multiple regression			
	ρ	p	β	95 % CI		p
FA (entire NAWM)	-0.39	**0.030**	-0.28	-0.58	0.03	0.071
FA (frontal NAWM)	-0.42	**0.020**	-0.29	-0.58	-0.01	**0.045**
FA (temporal NAWM)	-0.37	**0.041**	-0.25	-0.56	0.06	0.105
FA (cingulate NAWM)	-0.38	**0.034**	-0.33	-0.61	-0.04	**0.025**
RD (frontal NAWM)	0.36	**0.046**	0.30	0.00	0.59	0.052
RD (cingulate NAWM)	0.32	0.080	0.31	0.01	0.60	**0.040**
MD (frontal NAWM)	0.36	**0.046**	0.27	-0.03	0.58	0.077

Shown are Spearman correlation coefficients (ρ), standardised multiple regression coefficients (β), 95% confidence intervals and p-values (p) in the GFAP (high) subgroup of MS patients. Significant p-values are bolded. Multiple regression models were adjusted with sex and treatment (no, 1st and 2nd line). AD = axial diffusivity; DTI = diffusion tensor imaging; FA = fractional anisotropy; MD = mean diffusivity; NAWM = normal appearing white matter; RD = radial diffusivity

(22.52 pg/ml vs. 23.11 pg/ml, p = 0.6). Median GFAP concentration was 98.7 pg/ml among female and 89.9 pg/ml among male MS patients with no statistically significant difference (p = 0.9). Similarly, there was no difference in the NfL levels between female and male MS patients (20.7 vs. 31.4 pg/ml, p = 0.054). There were differences between GFAP (high) and GFAP(low) subgroups: patients in the GFAP(high) subgroup had longer disease duration and higher EDSS as well as larger T2 lesion volume and higher serum NfL level (Table 1).

In the GFAP(high) subgroup serum GFAP levels correlated with DTI indices (Fig. 1, Table 2), but not with NAWM, cortical grey matter, T1 or T2 volumes (data not shown). High GFAP correlated with low FA both in the entire NAWM and in frontal, temporal, and cingulate NAWM. In parietal and occipital NAWM the correlation did not reach statistical significance. In addition, higher GFAP correlated with increased MD and RD in the frontal NAWM. In the entire MS cohort GFAP correlated positively with T2 lesion volume (ρ = 0.29, p = 0.024) and negatively with cortical grey matter volume (ρ = -0.25, p = 0.049). Among healthy controls there were no correlations between GFAP and DTI indices (data not shown).

Multiple regression modelling was performed to further evaluate the association between GFAP and DTI (Table 2). Similarly to the results of Spearman correlation analysis, higher GFAP associated with lower FA in frontal and cingulate NAWM. In addition, in the GFAP(high) subgroup GFAP associated positively with cingulate RD (Table 2) and negatively with NAWM volume (standardised multiple regression coefficients β = -0.30 (95% confidence intervals -0.58 - -0.02), p = 0.034). In the entire MS cohort GFAP associated positively with T2 lesion volume (β = 0.32 (0.07-0.57), p = 0.012) and negatively with cortical grey matter volume [β = -0.29 (-0.53 - -0.04), p = 0.023] and occipital AD [β = -0.31 (-0.58 - -0.03), p = 0.029].

4. Discussion

We report here an association between increased serum GFAP and DTI-measurable micro-damage in the NAWM tracts in MS. Our results corroborate the assumption that serum GFAP concentration reflects brain astrocytic function, which in turn can contribute to white matter tract damage. Higher GFAP correlated with decreased FA and increased MD and RD, which are considered to reflect myelin and axonal loss in the white matter of MS patients (Sbardella et al., 2013). In other neurodegenerative diseases astrogliosis has similarly been observed in areas of reduced FA (Cardenas et al., 2017). Reactive astrocytes may promote neuroaxonal damage in multiple ways. They recruit peripheral immune cells into the CNS and microglia to lesion area, and they produce neurotoxic inflammatory mediators (Dendrou et al., 2015; Ponath et al., 2018). On the other hand, under certain conditions, astrocytes may promote neuroprotection and tissue repair (Ponath et al., 2018).

The patient profile with higher EDSS and longer disease duration in the GFAP(high) subgroup supports the hypothesis that higher GFAP associates with more profound neuroaxonal damage leading to disease progression. The higher NfL level in the GFAP(high) group is in line with previous reports showing a correlation between serum GFAP and NfL (Abdelhak et al., 2018; Ayrignac et al., 2020; Högel et al., 2020). Interestingly, we have recently demonstrated an association between higher NfL levels and DTI metrics reflecting diffuse neuroaxonal damage (Saraste et al., 2021). Together with the previous results, the results from the present paper support the notion that elevated serum concentrations of GFAP and NfL may reflect ongoing diffuse pathology outside focal lesions. However, significantly higher NfL concentrations have also been observed in association with acute focal inflammation (Kuhle et al., 2019; Varhaug et al., 2018), and it is hence always imperative to consider the clinical status of the MS patient, and stage of the disease when interpreting the biomarker results. The effect of relapses on the serum or CSF GFAP levels is less clear (Ayrignac et al., 2020; Burman et al., 2014; Martínez et al., 2015; Norgren et al., 2004; Watanabe et al., 2019), and needs to be studied further in the future.

In conclusion, our work supports the use of serum GFAP as a biomarker for MS-disease associated astrocytopathy and diffuse white matter damage. In the future, simultaneous detection of a combination of biomarkers may provide important information about the distinct aspects of the CNS-related pathology of MS in the clinical follow-up of the patients.

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Declaration of Competing Interest

The Authors declare that there is no conflict of interest.

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CRediT authorship contribution statement

Maija Saraste: Investigation, Writing - original draft, Writing - review & editing. **Svetlana Bezukladova:** Investigation. **Markus Matilainen:** Data curation, Formal analysis. **Marcus Sucksdorff:** Investigation. **Jens Kuhle:** Methodology. **David Leppert:** Methodology. **Laura Airas:** Conceptualization, Funding acquisition, Supervision, Writing - review & editing.

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