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Serum growth differentiation factor-15, glial fibrillary acidic protein, and neurofilament light chain: Their link and role in Creutzfeldt-Jakob disease

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ABSTRACT

Background: Creutzfeldt-Jakob disease (CJD) is a rapidly progressive neurodegenerative disorder characterized by neuronal damage. Emerging biomarkers, such as serum neurofilament light chain (sNfL), glial fibrillary acidic protein (sGFAP), and growth differentiation factor-15 (sGDF-15), are currently being studied for their potential use in this disease.

Objectives: This study analyzes the levels of sNfL, sGFAP, and sGDF-15, as well as their relationships, in patients with CJD compared to healthy controls (HC).

Methods: A total of 19 CJD patients and 81 age- and sex-matched HCs were enrolled. Serum levels of sNfL and sGFAP were measured using ultrasensitive immunoassays, while sGDF-15 levels were assessed via ELISA. Statistical analyses included correlation analysis and analysis of covariance (ANCOVA) models.

Results: CJD patients showed significantly higher serum levels of sNfL and sGFAP compared to HCs ($p < 0{,}001$). sNfL levels were positively correlated with both sGFAP (Rho = 0,70; $p < 0{,}001$) and sGDF-15 (Rho = 0,60; $p = 0{,}004$). Interestingly, sGFAP levels were higher in female CJD patients compared to males ($p = 0{,}001$), while no significant difference in sNfL levels was observed between sexes.

Conclusions: In conclusion, this study explores the potential of sNfL, sGDF-15, and sGFAP as biomarkers in CJD patients. The higher levels of sNfL and sGFAP in CJD patients compared to healthy controls, along with the observed sex differences in sGFAP, highlight the need for further research into the interaction between astroglia and neurons in CJD, with a focus on sex as a key variable.

1. Introduction

Creutzfeldt Jacob Disease (CJD) is a rapid progressive dementia associated with the conversion of the normal prion protein (PrPC) into its misfolded form (PrP^{Sc}), which accumulates within neuronal cells, leading to intracellular spongiform changes and neuronal loss. Although the underlying pathophysiology remains incompletely understood and no disease-modifying therapies are currently available, emerging biomarkers are garnering significant attention due to their potential as disease surrogates and predictors of disease onset years in advance [1], facilitating preclinical enrollment in clinical trial and enabling biological monitoring.

Focus has been placed on neurofilament light chain (NfL), an intermediate filament component of neuronal cytoskeleton released in blood and CSF following neuroaxonal damage or neuronal death [2]. Additionally, glial fibrillary acidic protein (GFAP), an intermediate filament expressed in astrocytes that is crucial for cellular structure and functions, is released during astrogliosis and astrocyte damage [3].

Growth differentiation factor 15 (GDF-15) is a systemic molecule that has recently gained increasing importance in neurological disease. It acts as a bridge between the cellular stress response and various biological functions or pathogenic processes in which it is involved. GDF-15, a member of the transforming growth factor β superfamily, is expressed by multiple cell types and released in bloodstream from various organs, as well as into CSF from central nervous system in response to cellular stress, including hypoxia and mitochondrial dysfunction [4]. It is associated with functions such as promoting angiogenesis, enhancing hippocampal neurogenesis, improving synaptic activity, reducing cell apoptosis, and modulating the inflammatory response by inhibiting the NF-kB pathway [5].

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The aim here is to evaluate the serum NfL (sNfL), sGFAP and sGDF-15 levels and their reciprocal relationships in patients with CJD and in comparison, to a matched healthy control (HC) group.

2. Methods

2.1. Enrollment

We enrolled patients with probable CJD using current CDC criteria [6] that performed both CSF RT-QUIC and brain MRI, with a compatible medical history. Age, sex, and past medical history were collected. Patients with a history of infectious diseases in the previous 2 months were excluded.

Serum samples from age- and sex-matched HCs without any history of autoimmune, psychiatric, or neurological diseases were collected.

2.2. NfL and GFAP assay

sNfL and sGFAP concentrations were assessed in 81 HCs samples, and 19 CJD patients using the commercially available immunoassay kits for NfL and GFAP-SimoaTM assay Neurology 2-Plex B (GFAP, NfL) Assay Kit (Catalog #103520; Quanterix, Billerica, MA, USA). The assay was run on the semi-automated ultrasensitive SR-X Biomarker Detection System (Quanterix). Samples were diluted 1:4 and randomly distributed on plates. Quality control (QC) samples, provided with the kit, exhibit concentrations in the predefined range, and the coefficient of variance between plates was maintained below 10 %. All samples were analyzed in a blinded manner using alphanumeric codes. Diagnostic codes were revealed only after QC-verified NfL and GFAP concentrations were reported to the database manager. Concentrations were measured in pg/ml and documented in the database. The analyses were conducted at the laboratory of the Centre for Precision Medicine and Translation of the University of Siena, Italy.

2.3. GDF-15 Assay

GDF-15 levels in each CJD patient and HCs serum sample were assessed using the GDF-15 Human ELISA kit (Bio-Techne, USA R&D Systems, Inc.). The readings were obtained on an iMark Absorbance Microplate Reader (Bio-Rad), following the manufacturer's instructions. Samples were diluted at a ratio of 1:4 and randomly distributed on the plates. Concentrations were measured in pg/ml and recorded in the database.

2.4. Statistical analysis

An initial evaluation of demographic and biomarker features in our samples was performed. A descriptive analysis considering the median and 25th –75th percentiles is reported in Table 1. Normal distribution was assessed using the Shapiro-Wilk test and Log10 transformation was applied when the normality assumption was violated (Following published studies, https://doi.org/10.1136/jnnp-2022-329933).

Based on preliminary results obtained from an initial sample of 6 patients, an a priori one-tailed power analysis was conducted using G*Power to calculate the sample size. The a priori power analysis assumed a correlation coefficient of 0,6 for the alternative hypothesis, with an α level of 0,05 and a desired power of 0,8 (1 - β). The results indicated that a minimum of 15 participants would be needed to detect a statistically significant correlation with the specified parameters.

A value of $p < 0.05$ was considered significant. Analysis results and graphs were generated with Jamovi Software. (The Jamovi Project, 2021). Influences between serum NfL, serum GFAP, and GDF-15 in the cohort of patients with CJD were investigated using one-tailed Spearman's correlation considering our preliminary results and according to the positive relationship already suggested by existing literature [7].

While analysis of covariance models (ANCOVA) was employed to investigate the impact serum GFAP, NfL and GDF-15 biomarker levels within and between the HC and CJD cohorts, with age and sex as covariates. A post-hoc pairwise comparisons with Bonferroni adjustment was performed to confirm significant results.

Table 1
Descriptive analysis of patients and biomarker data. GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; GDF-15, Growth differentiation factor 15.

	CJD cohort	HC cohort
SEX F/tot.	8/19	48/81
Age (median, 25th Percentile – 75th percentile) (years)	68,3 (61–74)	63 (54–72)
sNfL (median, 25th Percentile – 75th percentile) (pg/ml)	137,29 (92,78-268,18)	10,88 (7,5-15,96)
sGFAP (median, 25th Percentile – 75th percentile) (pg/ml)	684,98 (438,41-1926,53)	100,89 (49,93-168,76)
GDF-15 (median, 25th Percentile – 75th percentile) (pg/ml)	970 (671–355)	516 (374–818)
Log10NfL (median, 25th Percentile – 75th percentile)	2,14 (1,96-2,43)	1,04 (0,88-1,20)
Log10GFAP (median, 25th Percentile – 75th percentile)	2,84 (2,64-3,28)	2 (1,70-2,23)
Log10GDF-15 (median, 25th Percentile – 75th percentile)	2,99 (2,82-3,26)	2,71 (2,57-2,91)

3. Results

In our study, we enrolled a cohort of 81 HCs and 19 CJD patients. HC cohort had a median age of 63 years (25th–75th percentile: 54-72 years) and included 48 females and 33 males, while, CJD cohort controls had median age of 68,3 years (25th–75th percentile: 61–74 years), consisting of 8 females and 11 males.

No significant difference between HC and CJD cohort was detected in relation to age ($p = 0{,}107$) and sex ($p = 0{,}175$).

By correlation analysis, a significant positive correlation was found in CJD cohort between Log_{10}NfL and Log_{10}GDF-15 [$p < 0{,}004$; Rho spearman 0,60] **(**Fig. 1**, A)**, and Log_{10}NfL and Log_{10}GFAP [$p < 0{,}001$; Rho spearman 0,70] while no correlation was found between Log_{10}GFAP and Log_{10}GDF-15 [$p = 0{,}150$; Rho spearman 0,273].

With ANCOVA models, Log_{10}GFAP, Log_{10}NfL and Log_{10}GDF-15 values were compared between the CJD cohort and HC cohort, and the analysis revealed significantly higher Log_{10}GFAP [$p < 0{,}001$; $F(1, 95) = 134{,}34$; $\eta^2 p = 0{,}59$] and Log_{10}NfL [$p < 0{,}001$; $F(1, 95) = 252{,}84$; $\eta^2 p = 0{,}73$] values in the CJD cohort, while no difference were found between two cohorts in Log_{10}GDF-15 [$p = 0{,}513$] (Fig. 1**-B, C, D)**. Additionally, in the same ANCOVA model significantly higher Log_{10}GFAP values [$p = 0{,}001$; $F(1, 97) = 10.80$; $\eta^2 p = 0{,}10$] were found in females CJD, a post-hoc pairwise comparisons with Bonferroni adjustment confirmed the significant differences between males and females within CJD cohort [p bonferroni = 0,030, d Cohen = − 1,34] while no significant sex differences were found in Log_{10}NfL values **(**Fig. 1**- E, F)**.

4. Discussion

In this cross-sectional cohort study, we found within CJD cohort a significant correlation between levels of sNfL and sGDF-15, and an increased levels of sNfL and sGFAP in CJD patients in comparison with HCs. Interestingly, sGFAP levels were higher in female CJD patients compared to male patients.

sGDF-15 levels have been demonstrated to be strongly associated with the degree of neuronal damage, as previously described [7] in patients affected by Alzheimer's disease (AD). GDF-15 has been proposed to take part in the pathogenesis of CJD through the enhancement of neuroinflammation and mitochondrial and oxidative stress response dysfunction [8,9].

In relation to the first mechanism, elevated levels of interleukin (IL)-

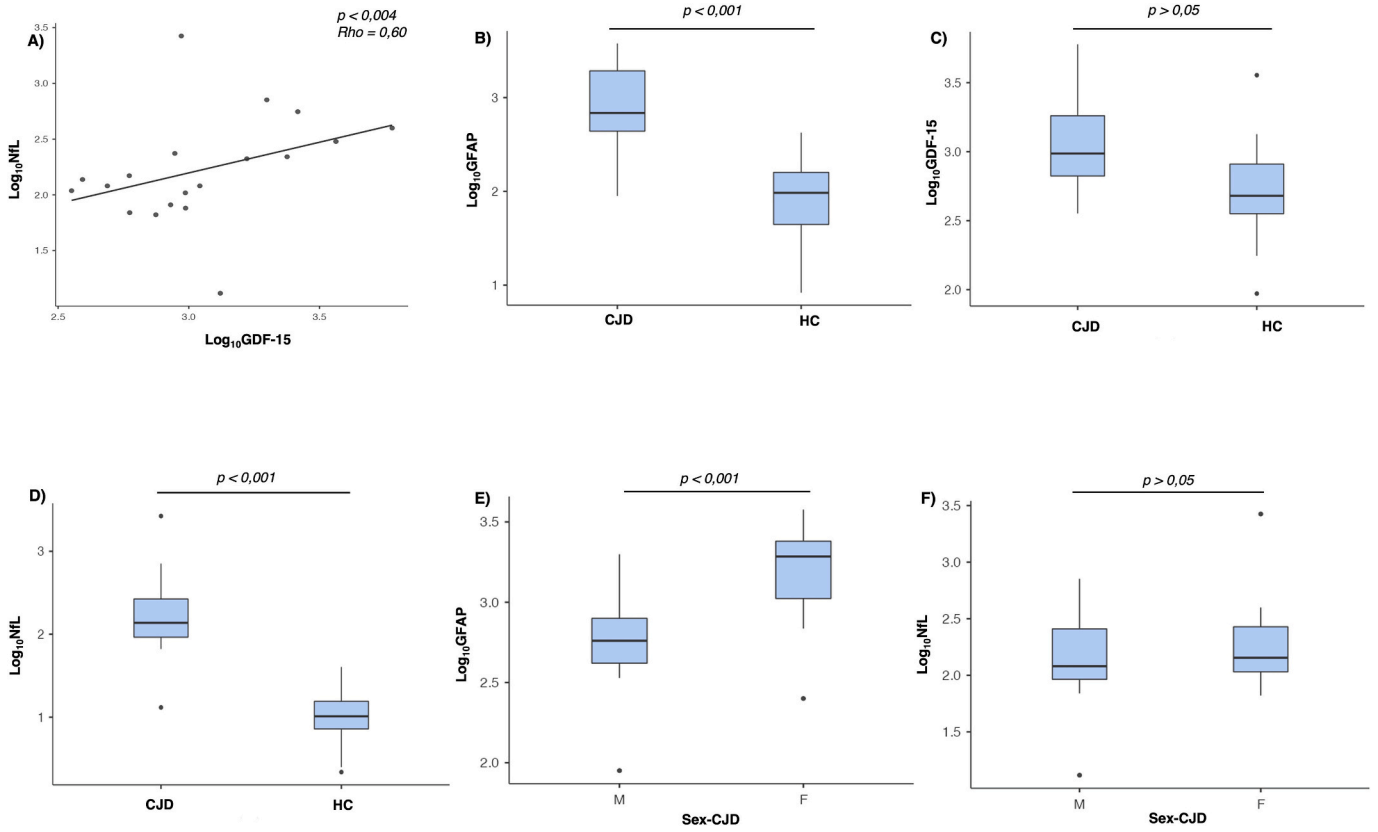

Fig. 1. Scatterplot of Log_{10}NfL-Log_{10}GDF-15 correlation. Box plots express the first (Q1) and third (Q3) quartiles by the upper and lower horizontal lines in a rectangular box, in which there is a horizontal line showing the median. The whiskers extend upwards and downwards to the highest or lowest observation within the upper (Q3 + 1.5 × IQR) and lower (Q1–1.5 × IQR) limits. *P* values indicate statistical significance between the different groups; A) Statistically significant correlation between of Log_{10}NfL-Log_{10}GDF-15 values, B) Statistically significant differences of Log_{10}GFAP values between two cohorts, C) No statistically significant difference of Log_{10}GDF-15, D) statistically significant differences of Log_{10}NfL values between two cohorts, E) Statistically significant comparison of Log_{10}GFAP values between males and females in CJD Cohorts, F) No statistically significant comparison of Log_{10}NfL values between males and females in CJD Cohorts.

6 [10], IL-4 and IL-10 [11] have been reported in CJD. Notably, IL-10 and IL-4 stimulate macrophages to release GDF-15, which plays a pivotal role in modulating inflammatory processes, particularly by promoting an anti-inflammatory phenotype [12].

In parallel, mitochondrial damage [13] is suggested by the decreased expression of some mitochondrial protein, including mitochondrial pyruvate carrier 1 and mitochondrial uncoupling protein 5 [14]. This process can be promoted by PrP^{sc} involving metal homeostasis, [9,15] through the reduced binding capacity of copper which impairs the cellular redox balance [9].

The elevation of sNfL levels detected in our CJD cohort was not unexpected given the robust evidence of a rapid mechanism of neuronal damage and death already well-documented in the literature [16–19]. Indeed, similar results were shown for NfL serum in other recent studies [20–23] and the use of NfL cut-off limits have also been proposed in the diagnosis of CJD [23,24].

On the contrary, limited evidence is currently available regarding sGFAP in CJD. This protein has been more studied in the CSF of CJD patients and is currently viewed as a good biomarker of the neuroinflammatory mechanisms characterizing CJD pathogenesis [20,21]. Interestingly, the presence of reactive astrocytes and astrogliosis is a characteristic neuropathological feature of prion diseases [25]. Immunohistochemistry and Western blot analyses of lysed human tissue have demonstrated increased GFAP protein expression [25,26], particularly near prion aggregates [27]. The presence of misfolded prion protein has been associated with astrocytic polarization towards a neurotoxic A1 phenotype [28] and an alteration of the function of astrocytes, which is an integral component of the associated neuroinflammatory process [27]. Glial activation is an early event in disease pathogenesis and persistent throughout the disease [29]. Although initially recognized as a response to neuropathological changes, glial cells contribute actively to neurodegeneration by inducing or exacerbating neuronal death through their role in the neuroinflammatory response [27].

In the CJD cohort, we found no differences in GDF-15 levels con comparison to those of HC. This result is analogous to what occurs in AD, where the levels of this biomarker do not differ in plasma or CSF compared to HC [30,31]. The interpretations of this lack of difference are still under debate; however, it may be because the regulation of this mitokine expression is more complex than expected. One hypothesis could be that neuronal expression of GDF-15 might serve as a better biomarker than the presence of this protein in biological fluids [31].

Another interesting observation comes from differences between males and females in sGFAP levels in CJD cohort in the absence of significant differences in sNfL values. Probably the hypothesis of sex differences in astrocytic activation, already shown in other neurological diseases including traumatic brain injury [32] and AD [33,34]. Although the causes remain elusive, an influence of ovarian hormones was proposed [35].

In conclusion this study started to explore the potential significance of sNfL, sGDF-15 and sGFAP as biomarkers in CJD patients. The elevated levels of sNfL and sGFAP compared to HCs, together with the observed sex differences in sGFAP suggest that the characterization of the interplay between the astroglia and neurons in the contest of CJD warrants further studies, in which sex should be considered as an important variable.

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CRediT authorship contribution statement

Carlo Manco: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Domenico Plantone:** Writing – review & editing, Supervision, Resources, Funding acquisition, Data curation, Conceptualization. **Delia Righi:** Supervision, Resources, Methodology, Formal analysis. **Sara Locci:** Methodology. **Sabina Bartalini:** Resources. **Roberto Marconi:** Visualization, Resources. **Nicola De Stefano:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

All the Authors have no competing interests in relation to this study.

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