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Investigating plasma neurofilament light chain levels in patients with migraine: a cross-sectional study

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ABSTRACT

Background: Migraine is a complex neurovascular disorder that extends beyond recurrent pain attacks. Although migraine is primarily considered a disorder of neuronal dysfunction, accumulating neuroimaging and experimental evidence suggests subtle structural and molecular brain alterations. Neurofilament light chain (NfL) is a well-established biomarker of axonal injury and neurodegeneration, but its role in migraine remains unclear. The present study aimed to compare plasma NfL levels between adults with migraine and healthy controls, and to explore potential associations with clinical and demographic characteristics.

Methods: This study included patients with migraine fulfilling the International Classification of Headache Disorders (ICHD-3) criteria and a group of healthy subjects as controls. Plasma NfL concentrations were quantified using the fully automated Lumipulse® immunoassay platform (Fujirebio, Gent, Belgium). Associations between plasma NfL levels and demographic and clinical characteristics were analyzed using correlation analyses and regression models adjusted for relevant confounders.

Results: A total of 60 patients with migraine (42 with chronic migraine [CM], 70%) and 22 healthy controls were enrolled. Mean plasma NfL levels did not differ between migraine patients and controls (11.10±4.96 pg/mL *vs.* 11.20±6.00 pg/mL, respectively; p=0.935) or between episodic and CM subgroups. Moreover, no significant correlations were observed between NfL levels and age, monthly migraine days (MMD), migraine-related disability, pain intensity, aura, medication-overuse headache (MOH), or acute medication intake.

Conclusions: Migraine, including its chronic form, was not associated with detectable neuroaxonal damage based on circulating NfL levels, suggesting that neuroaxonal injury is not a prominent feature of the disease. Larger longitudinal studies are warranted to further clarify the role of neuroaxonal biomarkers in migraine pathophysiology.

Key words: migraine, neurofilament light chain, axonal injury, biomarkers, neurodegeneration.

Introduction

Migraine is a highly prevalent and disabling neurological disorder characterized by recurrent headache attacks commonly accompanied by nausea and/or vomiting, photophobia, and phonophobia. (1,2) It may be preceded or accompanied by transient focal neurological symptoms, known as "aura". (2) According to the third edition of the International Classification of Headache Disorders (ICHD-3), chronic migraine (CM) is defined as headache occurring on ≥15 days/month for >3 months, with migraine features on at least 8 days/month. (2) Longitudinal studies indicate that approximately 2.5% of individuals with episodic migraine (EM) progress to CM annually, while CM may remit to EM, with a reported 2-year transition rate of about 26%. (3)

Although migraine is primarily considered a disorder of recurrent and potentially reversible neuronal dysfunction, interest in biomarkers of neural injury has increased in parallel with evidence of subtle central nervous system (CNS) alterations. (4,5) Neuroimaging studies have reported white matter lesions and MRI-derived volumetric changes in both white and gray matter. (6,7) However, these findings are heterogeneous and should not be interpreted as direct evidence of progressive neurodegeneration.

In parallel, fluid biomarkers have increasingly been investigated as potential indicators of neural injury in migraine. (4) S100 calcium-binding protein B (S100B) and neuron-specific enolase (NSE), two established biomarkers of CNS injury, have been investigated in migraine, but with inconsistent results. Studies have reported altered serum levels in both EM and CM, with some suggesting increased S100B and reduced NSE, and others showing greater NSE elevations in CM. (8,9) Furthermore, Overeem *et al.* reported elevated serum total tau (t-tau) levels in both EM and CM, whereas another study found increased serum t-tau only during the headache phase. (5,10) These heterogeneous findings highlight the need for more robust and reproducible biomarkers to clarify whether migraine is accompanied by measurable neuroaxonal injury. Neurofilament light chain (NfL), a key cytoskeletal component of large myelinated axons, has emerged as a gold-standard indicator of axonal injury. Unlike traditional markers, peripheral NfL levels provide a highly sensitive reflection of even minor neuroaxonal damage across a wide spectrum of neurological conditions, from multiple sclerosis (MS) to neurodegenerative diseases. (11,12) In multiple sclerosis, cerebrospinal fluid and serum NfL levels reflect disease activity and decrease following disease-modifying therapies. Elevated NfL concentrations have also been consistently reported in neurodegenerative disorders such as Alzheimer's disease, frontotemporal dementia, and amyotrophic lateral sclerosis, supporting their diagnostic and prognostic value. (12) Considering these preliminary data,

several studies have investigated whether plasma NfL concentrations differ between individuals with migraine and healthy controls; however, findings have been inconsistent and remain inconclusive. For instance, Fang *et al.* reported no significant overall difference in serum NfL levels between patients with migraine and controls, but identified higher concentrations in those with a disease duration of at least 10 years, particularly among individuals younger than 45 years. (4) By contrast, another case-control study (REFORM), found no significant difference in serum NfL concentrations between the two groups. (13) Similarly, a further multicenter case-control study, including 58 patients with CM and 69 controls, found no significant differences in NfL levels, supporting the view that CM is not typically associated with measurable structural neuroaxonal damage. (14)

These inconclusive findings highlight a critical gap in our understanding. It remains unclear whether these observations indicate a true absence of axonal damage or instead reflect the influence of clinical heterogeneity and confounding factors. Therefore, the present study aimed to compare NfL levels between a well-characterized cohort of adults with migraine and healthy controls, further exploring the influence of specific demographic and clinical phenotypes on this biomarker profile.

Results

Study population. A total of 60 adult patients with a diagnosis of migraine were enrolled. Among them, 18 were classified with EM (mean age 42.0±15.5 years; 72.2% female), while 42 patients were diagnosed with CM (mean age 44.4±13.1 years; 81.0% female). Twenty-two healthy controls were included (mean age 53.50±12.78 years; 68.2% female). Migraine with aura was present in 17/60 patients (28.3%), whereas 43/60 patients (71.7%) were classified as migraine without aura. Aura was present in 7/18 patients with EM and 10/42 patients with CM (p=0.276). Medication-overuse headache (MOH) was present in 32 patients with CM (53.3%).

NfL levels. Mean plasma NfL concentrations did not significantly differ between patients with migraine and healthy controls (11.10±4.96 pg/mL *vs.* 11.20±6.00 pg/mL, respectively; p=0.935). Given the borderline between-group difference in age (p=0.052), all statistical analyses were adjusted for age. Similarly, no significant differences in NfL levels were observed between patients with EM and CM (10.87±5.32 pg/mL *vs.* 11.18±4.87 pg/mL, respectively; p=0.833). **Figure 1** shows plasma NfL levels across diagnostic groups. **Table 1** summarizes the demographic and clinical characteristics of the study participants, together with plasma NfL levels.

Association between NfL levels and clinical variables. Correlation analyses revealed no significant associations between plasma NfL levels and demographic or clinical characteristics, including monthly migraine days (MMD),

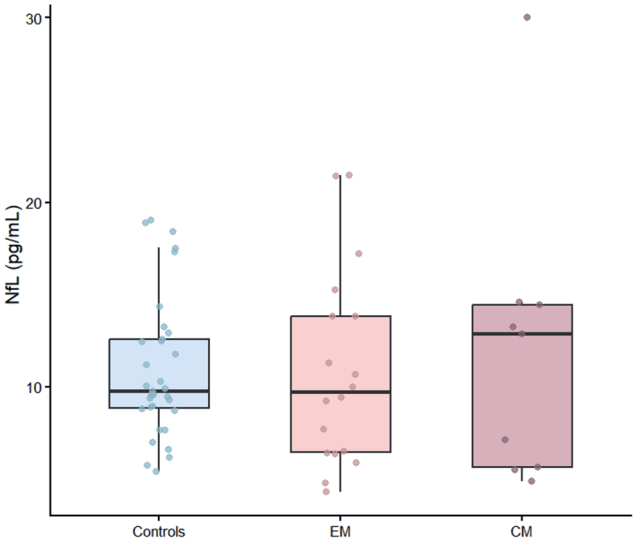

Figure 1. Box plot of plasma NfL concentrations (pg/mL) in healthy controls, EM, and CM groups.

Table 1. Demographics and clinical characteristics of the study participants and healthy controls.

	Patients		Control		p-value	CM		EM		p-value
	n	mean±SD or n (%)	n	mean±SD or n (%)		n	mean±SD or n (%)	n	mean±SD or n (%)	
Age	60	47±13.8	22	53.5±12.8	0.052	42	44.4±13.1	18	42.0±15.5	0.385
Sex	60	47 (78.3%)	22	15 (68.2)	0.383	42	34 (80.9)	18	13 (72.2)	0.490
Duration	58	31.0±14.4				40	29.8±15.6	18	31.6±14.0	0.756
NfL	60	11.10±4.96	22	11.2±6.0	0.935	42	11.2±4.9	18	10.9±5.3	0.833
MMD	59	20.3±8.4				41	24.7±5.9	18	10.2±2.1	0.0001
MIDAS	58	92.0±51.6				40	105±54.1	18	63.7±31.4	0.004
Aura	60	17 (28.3)				42	10 (23.8)	18	7 (38.9)	0.276
MOH	60	32 (53.3)				42	32 (76.2)	18	0 (0.0)	<0.001
Triptan intake	51	26 (51.0)				33	16 (48.5)	18	10 (55.6)	0.771
NSAID intake	51	34 (66.7)				33	23 (69.7)	18	11 (61.1)	0.551
Medication use	59	24.8±22.6				41	30.6±24.9	18	11.4±3.42	<0.001
VAS	49	8.1±1.3				36	8.1±1.35	13	8.1±1.2	0.962

CM, chronic migraine; EM, episodic migraine; SD, standard deviation; NfL, neurofilament light chain; MMD, monthly migraine days; MIDAS, Migraine Disability Assessment; MOH, medication overuse headache; NSAID, non-steroidal anti-inflammatory drug; VAS, Visual Analog Scale.

migraine-related disability (Migraine Disability Assessment; MIDAS), or migraine attack severity (Visual Analog Scale [VAS]). A weak positive trend toward an association between plasma NfL levels and disease duration was observed (r=0.252, p=0.056), although this did not reach statistical significance. A subgroup analysis stratified according to disease duration (≥10 years) showed no significant differences in NfL levels. In our cohort, no significant correlation was detected between NfL and age; similar results were obtained after log-transformation of NfL values (r=0.190, p=0.087).

Additional subgroup analyses showed no significant differences in plasma NfL levels according to MOH status (*t*-test p=0.847; Wilcoxon p=0.744) or aura status (*t*-test p=0.905; Wilcoxon p=0.789). Finally, NfL levels were not associated with triptan intake, non-steroidal anti-inflammatory drugs (NSAID) intake, or monthly medication use.

***Post-hoc* and regression analyses.** *Post-hoc* analyses confirmed the absence of significant differences in plasma NfL concentrations across diagnostic groups. Furthermore, linear and logistic regression models did not identify plasma NfL levels as a significant predictor of migraine diagnosis or disease chronicity after adjustment for potential confounders, including age, sex, and body mass index.

Discussion

In this cross-sectional study, plasma NfL concentrations did not differ significantly between migraine patients and controls, nor between EM and CM subgroups. Moreover, NfL levels were also not associated with migraine frequency, migraine-related disability, pain intensity, age, aura, MOH, acute medication intake, or disease duration. Overall, these findings do not support the presence of detectable neuroaxonal injury as reflected by circulating NfL levels. The absence of increased NfL levels in migraine patients aligns with the growing body of evidence suggesting that, despite its substantial clinical burden, the disorder does not appear to be associated with progressive neurodegeneration. NfL is a highly sensitive biomarker of axonal damage, widely used in neurodegenerative, inflammatory, vascular, and traumatic neurological disorders, where even subtle and subclinical axonal injury can be detected in blood. (11,12)

Our results are consistent with the hypothesis that migraine is not typically associated with cumulative axonal damage detectable in peripheral blood, even in chronic forms or among patients with greater disease burden. Our findings are in line with those of the REFORM study, (13) a large case-control investigation that found no differences in serum NfL levels between migraine patients and healthy controls, as well as with a recent multicenter study focusing on CM. (14) In contrast, a smaller monocentric study conducted in China reported a non-significant trend toward higher serum NfL levels in migraine patients, with subgroup analyses suggesting an association between longer disease duration and higher NfL concentrations, particularly in younger individuals. (4) Differences in study design, patient selection, and analytical platforms may partly explain these discrepancies. Importantly, our study used a fully automated and standardized chemiluminescence enzyme immunoassay (CLEIA)-based platform (Lumipulse®, Fujirebio, Gent, Belgium), thereby minimizing analytical variability and enhancing measurement reproducibility.

The lack of association between NfL levels and migraine chronicity is clinically relevant. CM is often considered a more severe disease state, characterized by central sensitization, altered pain processing, and higher disability. Neuroimaging studies have shown functional and structural brain changes in CM, including alterations in pain-related networks and an increased prevalence of white matter lesions. (15,16) However, our data suggest that these changes are unlikely to reflect ongoing axonal breakdown or neurodegeneration. Rather, they may reflect functional reorganization, adaptive plasticity, or non-destructive microstructural alterations, consistent with the absence of progressive neurological deficits in most patients with migraine. (16,17)

Similarly, we found no correlation between plasma NfL levels and MMD, MIDAS score, or pain intensity. These findings do not support a dose-response relationship between migraine burden and neuroaxonal injury. Although repeated attacks are associated with significant neuronal activation, metabolic stress, and neuroinflammatory signaling, such processes may not be sufficient to cause axonal damage detectable by NfL release into the bloodstream. (18) Alternatively, any transient axonal stress occurring during migraine attacks may be rapidly reversible and therefore not captured by a stable biomarker such as NfL in a cross-sectional design.

A non-significant trend toward an association between NfL levels and disease duration was observed in our cohort. Although this finding did not reach statistical significance, it may warrant further investigation in larger longitudinal samples to determine whether long-standing disease exerts subtle cumulative effects that remain below the detection threshold in smaller cohorts. Our study has several strengths, including the inclusion of both EM and CM patients, detailed clinical phenotyping, the use of a standardized analytical platform, and the assessment of multiple potential confounders through *post-hoc* and regression analyses.

Nevertheless, some limitations should be acknowledged. First, the cross-sectional design does not allow assessment of dynamic changes in NfL levels over time or during different phases of the migraine. In addition, blood sampling was not intentionally performed during migraine attacks, and exact timing relative to the last attack and acute medication intake was not systematically recorded for all participants. Second, although our cohort was clinically well characterized, the sample size, particularly for EM and healthy controls, may limit the detection of very small effect sizes. Despite adjustment for age, the slightly older age of the control group may still have influenced NfL comparisons. Third, only a single biomarker was assessed; therefore, NfL may not fully capture the biological complexity of migraine or other mechanisms such as neuroinflammation, synaptic dysfunction, or glial activation. Fourth, NfL is a non-specific marker of axonal injury and does not provide information on regional brain involvement; subtle, localized changes might escape detection in peripheral blood. Future longitudinal studies are warranted to evaluate whether plasma NfL levels fluctuate during acute migraine attacks, over long-term disease evolution, or in response to preventive treatments. The integration of blood biomarkers with advanced neuroimaging and neurophysiological measures may further clarify whether migraine-related brain alterations reflect functional plasticity rather than structural damage.

Conclusions

In conclusion, in this single-center cross-sectional cohort, plasma NfL levels did not differ between patients with migraine and healthy controls, nor between EM and CM. These findings do not support the presence of ongoing neuroaxonal injury detectable by plasma NfL in our cohort. However, given the relatively small sample size, the cross-

sectional design, and the assessment of a single biomarker, these results should be interpreted with caution.

By reinforcing evidence of no significant differences in circulating NfL between individuals with migraine and healthy controls, our study contributes to the growing literature indicating that migraine does not entail measurable neuroaxonal damage. However, larger longitudinal investigations are warranted to further elucidate the molecular and neurobiological mechanisms of migraine and to clarify the potential role of emerging biomarkers in refining disease characterization and prognosis.

Materials and Methods

Study design and population. In this single-center, cross-sectional study, we enrolled consecutive patients with migraine attending our Headache Clinic between June 2024 and September 2025. Inclusion criteria were: i) age ≥18 years; and ii) fulfillment of ICHD-3 criteria for migraine. Patients were classified as suffering from EM or CM, according to the criteria reported previously (ICHD-3 2018). A cohort of healthy controls, without a history of migraine, was also recruited. For this study, we excluded all participants who reported comorbid neurological disorders and recent traumatic brain injury, conditions likely to influence plasma NfL. At baseline, demographic and clinical characteristics were collected, including MMD, MIDAS, and VAS. Headache Impact Test-6 was not included in the main analyses because it was missing in most cohort members. Monthly headache days were not systematically available; therefore, MMD and monthly medication use were reported.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee (Comitato Etico Interaziendale, AOU Città della Salute e della Scienza di Torino, protocol number 0081225, July 2022). Written informed consent was obtained from all participants prior to enrollment.

Sample collection and laboratory methods. Peripheral venous blood samples were collected under standardized conditions, into ethylenediaminetetraacetic acid (EDTA) tubes. Blood sampling was not intentionally performed during migraine attacks. Samples were collected during routine clinical evaluation under standardized conditions. Plasma was separated by centrifugation (2000 × g for 10 min at room temperature) and aliquoted into polypropylene vials before storage at −80°C. Plasma NfL concentrations were measured using the Lumipulse® G1200II platform, a fully automated chemiluminescent enzyme immunoassay. The Lumipulse G NfL assay (ref. 231456) was used according to the manufacturer's instructions. NfL values were expressed in pg/mL.

Statistical analysis. Statistical analyses were performed using R (version 4.5.1). Continuous variables were tested for normality using the Shapiro-Wilk test and are presented as mean ± standard deviation; categorical variables are reported as counts and percentages.

Comparisons of plasma NfL concentrations between migraine patients and healthy controls, as well as between EM and CM subgroups, were conducted using appropriate parametric or non-parametric tests. Additional subgroup analyses were performed according to MOH status, aura status, triptan intake, and NSAID intake. Pearson's correlation analysis was used to assess associations between NfL levels and demographic or clinical variables, including monthly medication use. *Post-hoc* analyses, including linear and logistic regression models, were performed to evaluate the independent contribution of NfL to migraine status while adjusting for potential confounders. A two-tailed p-value <0.05 was considered statistically significant.

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Availability of data and materials: the data presented in this study are not publicly available due to privacy and ethical restrictions.

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