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Research report

Neural damage and inflammation in myotonic dystrophy type 1: Longitudinal analysis of serum NFL, GFAP, and IL-6

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ABSTRACT

Introduction: Myotonic dystrophy type 1 (DM1) is a progressive, multisystemic disease affecting the central nervous system (CNS). Blood-based biomarkers such as neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP), and interleukin-6 (IL-6) offer potential as non-invasive indicators of CNS dysfunction and/or inflammation. However, their longitudinal dynamics and clinical relevance in DM1 remain unclear. Additionally, sex-related differences in these biomarkers are poorly understood. This study aimed to investigate NFL, GFAP, and IL-6 serum levels in patients with DM1, examine sex-differences, track changes over four years, and explore associations with genetic, muscular, cognitive, and neuroimaging outcomes.

Method: Retrospective data from 70 DM1 patients and 54 healthy controls (HC) were analyzed. Longitudinal data were available for 68 participants (39 DM1, 29 HC). Biomarkers were measured using the ELLA immunoassay. DM1 patients had data on genetic, muscular, cognitive and structural brain outcomes. Analyses were adjusted for age.

Results: NFL and IL-6 levels were significantly higher in DM1 patients compared to HC, while GFAP levels did not differ. Male DM1 patients exhibited higher NFL and IL-6 levels compared to females. No significant longitudinal changes were observed over a four-year period. NFL and IL-6 levels correlated with larger genetic expansions and poorer cognitive performance.

Discussion: NFL and IL-6 may reflect neural damage and systemic inflammation in DM1 and could serve as biomarkers of cognitive dysfunction. However, their limited longitudinal sensitivity suggests longer follow-up is needed to evaluate their utility for disease monitoring.

1. Introduction

Myotonic dystrophy type 1 (DM1) or Steinert's disease is a progressive, hereditary neuromuscular disease transmitted in an autosomal dominant manner. It is caused by a trinucleotide cytosine-thymine-guanine (CTG) repeat expansion in the *DMPK* gene (Depienne and Mandel, 2021; Paulson, 2018). A clear genotype-phenotype correlation is typically observed: larger CTG expansions are associated with earlier disease onset and greater disease severity (Bigot et al., 2009; Harper, 2001; Hunter et al., 1992). Nonetheless, DM1 presents with considerable phenotypic variability, spanning a wide range in age of onset, symptom profile, severity, and progression.

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In addition to classical muscular involvement, DM1 has a significant impact on the central nervous system (CNS). Common CNS-related symptoms include fatigue, apathy, hypersomnolence, cognitive deficits, and structural and functional brain alterations (Simoncini et al., 2020). Importantly, these changes can manifest early in the disease, even before the onset of muscular symptoms, as seen in the Premanifest DM1 subgroup. These individuals have a confirmed genetic diagnosis but lack muscular impairment. For instance, evidence shows that Premanifest DM1 patients exhibit brain structural vulnerability, such as white matter (WM) tract disintegration (Plas et al., 2021), reduced grey matter (GM) volume, and white matter lesions (WML) (Garmendia et al., 2023). Preliminary data also indicate elevated levels of neural damage biomarkers in the blood of these individuals (van der Plas et al., 2022).

The observation of early cognitive and brain changes suggest an atypical neurodevelopmental trajectory in DM1 (Garmendia et al., 2023; Angeard, 2019; Axford and Pearson, 2013; De Serres-Bérard et al., 2021; Labayru et al., 2020a; Van Der Plas et al., 2019). Beyond this neurodevelopmental disruption, longitudinal studies point to a slow but progressive neurodegenerative component, evidenced by brain atrophy (Labayru et al., 2020a; Cabada et al., 2021; Conforti et al., 2016; Koscik et al., 2023; Labayru et al., 2022) and cognitive decline (Cabada et al., 2021; Fujino et al., 2023; Gallais et al., 2017; Gliem et al., 2019; Labayru et al., 2020b; Minnerop et al., 2018; Modoni et al., 2008; Sansone et al., 2007; Winblad et al., 2016). Combined with recent clinical (Garmendia et al., 2024a) and cellular and molecular findings (García-Puga et al., 2022; Hasuike et al., 2022; Mateos-Aierdi et al., 2015; Meinke et al., 2018), this evidence supports the view of DM1 as a model of accelerated aging, where atypical neurodevelopment may predispose patients to gradual neurodegeneration over time.

Neuropathological studies in DM1 have identified protein and nucleotide deposits, neuronal and glial changes, and white matter alterations (Weijs et al., 2021). However, many of these changes are not specific to DM1 and can overlap with those seen, to a lesser extent, during normal aging. In this context, recent advances in blood-based CNS biomarkers have attracted growing interest due to their potential clinical utility. These biomarkers are minimally invasive, cost-effective, and can be easily integrated into routine practice, making them promising tools for elucidating disease mechanisms and tracking treatment outcomes (Mapstone et al., 2020). However, to get this potential, it is critical to establish their relationship with CNS function, cognition, and key disease characteristics in DM1. Moreover, their value as predictors of disease progression must be rigorously evaluated.

Among these biomarkers, neurofilament light chain (NFL) – a neuronal protein expressed in axons – has consistently been identified as a sensitive marker of neural damage across various neurodegenerative diseases, and also in DM1, where elevated NFL levels have been observed (van der Plas et al., 2022; Laforce et al., 2022; Nicoletti et al., 2022; Rossi and Silvestri, 2023; Saak et al., 2021). Increased NFL has also been linked to poorer cognitive performance in other neurological conditions and older adults (Li et al., 2024; Meng et al., 2025; Ramani et al., 2021), but evidence for this association in DM1 remains scarce (Nicoletti et al., 2022).

Glial fibrillary acidic protein (GFAP), a protein primarily expressed in astrocytes and characteristic of astrogliosis, has been shown to enter peripheral circulation following CNS injury. Elevated GFAP levels have been reported in both Premanifest DM1 (van der Plas et al., 2022) and in DM1 more broadly (Laforce et al., 2022). However, data remain limited, and its relationship with cognitive performance and clinical features in DM1 has yet to be clearly established.

Inflammation — a central regulatory pathway related to cognitive and brain health— may also play a key role in DM1. Chronic activation of inflammatory pathways is associated with increased production of proinflammatory cytokines, which can lead to cellular damage, neuroinflammation, and cognitive decline (Mapstone et al., 2020). Although the evidence is still emerging, systemic immune dysregulation has been observed in DM1, with increased levels of proinflammatory markers such as tumor necrosis factor-alpha (TNF-α) and interleukins (IL-1, IL-6) (Azotla-Vilchis et al., 2021; Johansson et al., 2000; Mammarella et al., 2002; Nakamori et al., 2017). Among these, interleukin-6 (IL-6)—a multifunctional cytokine involved in both innate and adaptive immune responses—is particularly notable. IL-6 is a senescence-associated factor implicated in muscle wasting and atrophy (Muñoz-Cánoves et al., 2013). Beyond its peripheral effects, persistent IL-6 elevation may also contribute to neuroinflammation and CNS dysfunction in DM1, as well as to processes associated with accelerated aging.

To date, only one study has analyzed the longitudinal progression of neural damage biomarkers in DM1 (van der Plas et al., 2022), and to our knowledge, only one has investigated their association with cognitive function in this population (Nicoletti et al., 2022). However, critical gaps remain. Specifically, there is a need to clarify the relationship between these biomarkers and cognitive status, assess their validity as indicators of CNS injury, and determine their utility as markers of disease progression in DM1. Longitudinal biomarker studies are crucial for elucidating the natural history of the disease, informing clinical registries, and supporting the election of efficacy subrogate measures in the design of clinical trials.

Additionally, it remains unclear whether biomarkers of neural damage and inflammation differ between males and females. The available literature on sex-related differences in DM1 is limited and inconclusive. One study reported potential sex-related differences in the clinical presentation and severity of DM1 (Dogan et al., 2016), as well as in the risk of cancer (Fernández-Torrón et al., 2016), while others found no differences or patterns comparable to those observed in HC (Ouyang et al., 2019). Furthermore, the sex of the disease-transmitting parent also influences the intergenerational instability of the expansion and the disease expression (Brunner et al., 1993; Cobo et al., 1993; Lanni and Pearson, 2019), although further research in this area is still scarce. For instance, one study reported poorer cognitive performance in patients who inherited DM1 maternally (Garmendia et al., 2024b). Given these uncertainties, further research is needed to investigate sex-specific differences in CNS involvement in DM1. Future studies should consider both the sex of the patient and that of the transmitting parent to better understand disease heterogeneity and support the development of personalized treatment strategies.

The current study had four primary aims: (1) to assess blood-based markers of neural damage (NFL, GFAP) and inflammation (IL-6) in DM1 patients, including a focused analysis of the Premanifest DM1 subgroup; (2) to examine longitudinal changes in these biomarkers over a four-year period; (3) to investigate associations between biomarker levels and a range of demographic, clinical, molecular, cognitive, and neuroimaging outcomes in DM1; and (4) to explore potential sex-related differences in biomarker expression, taking into account both the sex of the patient and the sex of the transmitting parent (inheritance pattern).

2. Method

2.1. Participants

The sample comprised 124 participants, including 70 DM1 patients (57 Manifest DM1 and 13 Premanifest DM1) and 54 healthy controls (HC). Of these, 68 participants (39 DM1: 31 Manifest, 8 Premanifest; and 29 HC) had blood samples available at follow-up, with a mean follow-up interval of 4.22 years (SD = 0.41). Premanifest DM1 individuals were defined as those with a genetically confirmed DM1 diagnosis who did not present muscular symptoms, as measured by the Muscular Impairment Rating Scale (MIRS) score of 1. In contrast, Manifest DM1 individuals had a confirmed DM1 diagnosis and exhibited muscular symptoms (MIRS >1).

DM1 patients were recruited from the outpatient National Reference for Rare Neuromuscular Disorders Unit (CSUR) at the Neurology Department at Donostia University Hospital (Gipuzkoa, Spain). The inclusion criteria included being aged 18 or older and having molecular

confirmation of DM1. Exclusion criteria included a history of major psychiatric disorders, other neurologic disorders, or substance abuse. Patients with the congenital form of DM1 were excluded due to the distinct clinical profile of this form (Turner and Hilton-Jones, 2010). HC were recruited from non-affected relatives of DM1 patients (with a confirmed negative genetic test) and healthy volunteers. The inclusion and exclusion criteria for the HC group were the same as those for DM1 patients, except for the requirement of molecular confirmation of the disease. For the magnetic resonance imaging (MRI) assessment, participants with paramagnetic implants (e.g., pacemakers) were excluded for the MRI.

For the follow-up assessments conducted 4 years later, participants were contacted by phone and invited to continue with the study. Those who agreed were scheduled for an in-person visit at the hospital. Considering the risk of selective attrition bias in longitudinal studies, we conducted a comparative analysis of the study variables to assess equivalence, comparing DM1 patients who had only baseline data available (n = 32) because they abandoned the study, with those who had both baseline and follow-up data (n = 38).

This study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of the Gipuzkoa Health Area (DMRM-2017–01). All participants were informed about the study and provided written informed consent prior to their inclusion. Written consent was obtained again at the follow-up assessment.

2.2. Assessment

At both baseline and follow-up, blood samples were collected from DM1 patients and HC. Additionally, DM1 patients underwent clinical assessment, neuropsychological testing, and MRI only at baseline, to explore their correlations with biomarker levels.

2.2.1. Blood sample collection and analysis

Peripheral blood samples were collected from DM1 patients and HC at both baseline and follow-up at the hospital facilities. Samples were processed using standard protocols to separate cells, plasma, and serum, and to extract DNA. Aliquots were stored at −80°C at the Basque Biobank, located within the Biogipuzkoa Health Research Institute.

Serum samples were analyzed using the ELLA Automated Immunoassay System (ProteinSimple, Bio-Techne) to quantify levels of NFL, GFAP, and IL-6. All biomarkers were measured at both time points using predesigned assay plates specific to each marker, following the manufacturer's protocols (Bio-Techne). The following assay kits were used: Simple Plex Human NF-L Cartridge (Catalog Nº. SPCKB-PS-002448), Simple Plex Human GFAP (2nd Gen) Assay Cartridge (Catalog Nº. SPCKB-PS-009134), and Simple Plex Human IL-6 (2nd Gen) Assay Cartridge (Catalog Nº. SPCKB-PS-003028).

For statistical analyses, biomarker concentrations with a value of zero were retained and considered valid.

In DM1 patients, the size of the CTG repeat expansion was assessed at baseline for those without recent genotyping data (i.e., within the past five years). In all cases CTG expansion size was determined through genetic analysis of the *DMPK* gene isolated from circulating leucocyte DNA. For *DMPK* alleles containing up to approximately 100 CTG repeats, PCR was used, while Southern blot analysis was performed for larger expansions (Kamsteeg et al., 2012).

2.2.2. Clinical assessment

Muscular impairment in DM1 patients was evaluated at baseline by an experienced neurologist using the Muscular Impairment Rating Scale (MIRS) (Mathieu et al., 2001). This five-point scale assesses the severity of muscle weakness, progressing from distal to proximal regions: (1) no muscular impairment, (2) minimal signs, (3) distal weakness, (4) mild to moderate proximal weakness, and (5) severe proximal weakness. Patients with a confirmed genetic DM1 diagnosis who do not present with muscular symptoms (MIRS scale = 1) were classified as Premanifest DM1 individuals.

DM1 phenotype classification was based on the age of the first symptom onset, including childhood-onset (1–10 years), juvenile-onset (10–20 years), adult-onset (20–40 years), and late-onset (>40 years) phenotypes. Information on inheritance pattern—maternal or paternal transmission—was extracted from medical records.

2.2.3. Neuropsychological assessment

At baseline, DM1 patients completed a comprehensive neuropsychological evaluation designed to estimate intelligence quotient (IQ) and assess five core cognitive domains: attention/processing speed, memory, visuoconstruction, executive functions, and language.

A detailed description of the specific tests used to assess each domain is provided in the Supplementary Table 1). Raw scores from all neuropsychological tests were converted into standardized T-scores (mean = 50, SD = 10) using normative data from the Spanish population. IQ scores were standardized separately, with a mean of 100 and a standard deviation of 15.

2.2.4. Brain imaging (MRI)

Eighteen DM1 patients underwent MRI at baseline (2017) using a 1.5 Tesla scanner (Achieva Nova, Philips). Imaging included a high-resolution volumetric "turbo field echo" (TFE) sequence (sagittal 3D T1-weighted acquisition; TR = 7.2 ms, TE = 3.3 ms, flip angle = 8°, matrix = 256 × 232, slice thickness = 1 mm, voxel size = 1 × 1 × 1 mm, NSA = 1, 160 slices, no gap, total scan time = 5′34″).

Structural MRI data were analyzed using voxel-based morphometry (VBM). GM and WM brain tissue volumes were estimated with the FMRIB Software Library (FSL version 6.01) (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/). Standard preprocessing steps were applied (Ashburner and Friston, 2000), and global GM and WM volumes, normalized for head size, were calculated using SIENAX (Smith et al., 2002).

WMLs were assessed using the Wahlund scale for age-related WM changes, which were defined as hyperintense areas on T2-weighted images (Wahlund et al., 2001). Lesions larger than 5 mm were rated on a severity scale from 0 (no lesions) to 3 (diffuse involvement).

2.3. Statistical analyses

All statistical analyses were conducted using IBM SPSS Statistics version 28. The normality and homoscedasticity of outcome variables were assessed to guide the selection of appropriate statistical methods. As biomarker distributions (NFL, GFAP, IL-6) deviated from normality, non-parametric tests were applied. Descriptive statistics were computed, and chi-square tests were used for categorical variables.

Group comparisons were conducted to examine differences in biomarker levels (NFL, IL-6, and GFAP) between DM1 patients and HC. Additionally, comparisons were made between Premanifest DM1 and Manifest DM1 subgroups, as well as between Premanifest DM1 and HC. Additionally, sex-related differences were analyzed, including both the sex of the patient and that of the disease-transmitting parent, in biomarker levels and background variables (genetic, muscular, education, cognitive performance, and structural brain outcomes), adjusting for age.

Given that biomarker levels are known to be age-sensitive, all analyses were adjusted for age. To account for non-normality while controlling for covariates, Quade's Rank Analysis of Covariance—a non-parametric alternative to ANCOVA—was used. This method adjusts dependent variables for covariates (in this case, age), ranks the residuals, and then the Mann–Whitney *U* test is applied for group comparisons.

For longitudinal analyses, both intragroup and intergroup comparisons were conducted. Intragroup comparisons evaluated within-subject changes in biomarker levels from baseline to follow-up using the

Wilcoxon signed-rank test, a non-parametric method. Analyses were conducted separately for DM1 patients (Premanifest and Manifest) and HC. For intergroup comparisons, change scores (Δ values) were calculated for each biomarker. As in the cross-sectional analyses, Quade's Rank Analysis of Covariance was applied to adjust for the time interval between assessments. Group differences in biomarker change were then assessed using the Mann–Whitney *U* test on ranked residuals. Finally, as mentioned previously, additional analyses were conducted among DM1 patients to explore potential bias related to selective attrition, comparing those who discontinued participation with those who completed the follow-up.Spearman's correlation analyses were conducted in DM1 patients to examine the relationships between baseline biomarker levels (NFL, IL-6, GFAP) and clinical outcomes. Correlates included molecular measures (CTG repeats), muscular impairment (MIRS score), cognitive performance (IQ and various specific domains), and MRI-based brain metrics. All correlations were adjusted for age.

Additional analyses examined associations between biomarker levels and age in both DM1 and HC groups. To assess the stability and interrelationships of biomarker levels over time, correlations between baseline and follow-up values were also computed within each group.

Effect sizes for non-parametric tests were reported using the Rank-Biserial correlation coefficient (r_b) and interpreted as follows: small (r_b = 0.10–0.29), moderate (r_b = 0.30–0.49), and large ($r_b \geq 0.50$) (Cohen, 1988; López-Martín and Ardura, 2023). For Spearman's correlation analyses, effect sizes were reported and interpreted as small ($r_s \leq 0.19$), moderate ($r_s = 0.20$–0.29), and large ($r_s \geq 0.30$) according to López and Ardura's (2023) criteria (López-Martín and Ardura, 2023).

3. Results

3.1. Descriptive analyses

Of the 70 DM1 patients, 36 were female (51.4 %), and of the 54 HC, 35 were female (64.8 %). Sex distribution was equivalent between the groups (Chi-square contingency test, χ^2 (1) = 2.23; $p = 0.135$).

DM1 patients and HC were comparable in age at both baseline ($t = 1.21$ [94]; $p = 0.230$) and follow-up ($t = 1.99$ [70]; $p = 0.050$). The mean age at baseline for DM1 patients was 50.36 years (SD=10.43) and 53.13 years (SD=13.11) for HC. Within the DM1 group, baseline mean age was 47.91 years (SD = 8.85) for Manifest DM1 patients and 61.10 years (SD = 10.36) for Premanifest DM1 patients, a statistically significant difference ($t(68) = -4.70$, $p < 0.001$**).

In terms of DM1 phenotype distribution, 35 patients (50.0 %) were classified as having adult-onset, 19 (27.1 %) as juvenile-onset, 11 (15.7 %) as late-onset, and 5 (7.1 %) as childhood-onset. Regarding the inheritance pattern, 68.6 % of the DM1 sample had paternal inheritance, 27.1 % had maternal inheritance, 1.4 % (n = 1) inherited from both progenitors, and for the remaining 2.9 %, this information was not available.

Baseline descriptive data for the DM1 patient group, including demographic, molecular, clinical, cognitive, and brain structural outcomes, are summarized in the following table (Table 1). As shown, the cognitive profile of DM1 patients indicated a mean estimated IQ within the normal range, with average performance also observed across all assessed cognitive domains.

Regarding blood-based serum biomarkers, it is important to note that NFL and IL-6 levels were reliably detected using the ELLA platform, while GFAP levels were undetectable in a substantial number of samples (59.5 %), corresponding to values below the assay's detection limit (GFAP = 0). This likely reflects the low circulating levels of GFAP in peripheral blood within this cohort and/or limitations in the analytical sensitivity of the assay. Importantly, the proportion of undetectable values was similar for DM1 patients and HCs (60 % vs. 58.6 %, respectively).

3.2. Cross-sectional analyses of blood-based serum biomarkers

Results from the cross-sectional analyses are presented in Tables 2 and 3. Statistically significant findings are also visualized in Figs. 1 and 2 for clarity.

After adjusting for age, DM1 patients showed significantly higher levels of NFL (mean rank= 76.06 vs 44.93) and IL-6 levels (mean rank=75.37 vs 45.93) compared to HC, both at baseline and follow-up (see Fig. 1), with moderate to large effect sizes (NFL baseline, U= 942; $p < 0.001$**; r_b= 0.43; NFL follow-up, U= 240; $p < 0.001$**; r_b= 0.49; IL-6 baseline, U= 989; $p < 0.001$**; r_b= 0.41; IL-6 follow-up, U= 308; $p < 0.002$*; r_b= 0.38). In contrast, no significant group differences were observed in GFAP levels between DM1 patients and HC (Table 2).

Comparing Premanifest DM1 and Manifest DM1 patients (after adjusting for age) revealed significant differences only in IL-6 levels at baseline (mean rank 20.46 vs 38.93; U= 175; $p = 0.003$*; r_b= 0.35), but not at follow-up (mean rank 15.00 vs 20.52; U= 77; $p = 0.236$; r_b= 0.19). At baseline, patients with Manifest DM1 showed higher IL-6 levels (see Fig. 2). No significant differences were found in NFL and GFAP

Table 1
Baseline descriptive data of DM1 patients: demographic, clinical, molecular, cognitive, and brain outcomes.

	n (%)	*M* (SD)	Min-Max
Age	70	50.36 (10.43)	31.56–72.56
MIRS	70	2.54 (0.97)	1.00–4.00
MIRS 1	13 (18.6)		
MIRS 2	17 (24.3)		
MIRS 3	29 (41.4)		
MIRS 4	11 (15.7)		
MIRS 5	0 (0)		
CTG	65	660.20 (525.29)	63.00–1733.00
Years of education	70	14.84 (4.89)	5.00–25.00
IQ	64	95.25 (14.11)	60.00–126.00
Attention/PS	64	41.54 (7.41)	15.00–52.30
Memory	64	47.83 (8.81)	24.90–65.20
Visuoconstruction	64	44.69 (9.64)	23.00–70.00
Executive Functions	64	43.68 (7.18)	27.70–56.80
Language	64	50.92 (9.26)	30.00–69.00
GM vol	18	729055.36 (50266.10)	652350.92–807957.37
WM vol	18	676625.28 (34337.47)	612255.35–745762.69
WML	11	6.82 (5.76)	0.00–18.00

Note. DM1 = myotonic dystrophy type 1; *M*= mean; *SD*= standard deviation; n = sample; MIRS= muscular impairment rating scale; CTG= cytosine-thymine-guanine; IQ = intelligence quotient; PS= processing speed; GM=gray matter; WM = white matter; WML = white matter lesions. IQ scores are standardized ($M = 100$; $SD = 15$). Cognitive domain scores are presented as T-scores ($M = 50$; $SD = 10$). Brain volumes are shown in cm^3 after normalization. WMLs are rated using the Wahlund scale.

Table 2
Cross-sectional comparison of serum NFL, IL-6, and GFAP levels between DM1 patients and HC, adjusted for age.

	DM1			**HC**			***DM1 vs HC***		
	n	*Median (IQR)*	*Mean rank*	n	*Median (IQR)*	*Mean rank*	*U*	*p*	r_b
Baseline									
NFL	70	31.65 (18.88)	76.06	54	20.25 (15.90)	44.93	941	**< 0.001****	**0.43**
IL-6	70	4.06 (3.93)	75.37	54	1.84 (2.27)	45.81	989	**< 0.001****	**0.41**
GFAP	61	0.00 (4.44)	45.30	29	0.00 (7.78)	45.93	872	0.914	0.01
Follow-up									
NFL	39	27.30 (18.90)	42.85	29	18.20 (12.90)	23.28	240	**< 0.001****	**0.49**
IL-6	38	3.99 (4.31)	40.39	29	2.08 (1.55)	25.62	308	**0.002***	**0.38**
GFAP	31	0.00 (4.56)	20.92	10	0.00 (6.36)	21.25	153	0.939	0.01

Notes. DM1 = myotonic dystrophy type 1; HC = healthy controls; NFL = neurofilament light chain; IL-6 = interleukin-6; GFAP =glial fibrillary acidic protein. IQR = interquartile range; *U* = Mann-Whitney *U* test; *p* = *p*-value; r_b = rank-biserial correlation coefficient. *p < 0.05; **p < 0.001.

Table 3
Cross-sectional comparison of serum NFL, IL-6, and GFAP levels between Premanifest and Manifest DM1 patients, and between Premanifest patients and HC, adjusted for age.

	Premanifest			**Manifest**			***Premanifest vs Manifest***		
	n	*Median (IQR)*	*Mean rank*	*n*	*Median (IQR)*	*Mean rank*	*U*	*p*	r_b
Baseline									
NFL	13	37.00 (18.80)	32.54	57	31.20 (18.95)	36.18	332	0.561	0.07
IL-6	13	2.54 (3.98)	20.46	57	4.58 (4.12)	38.93	175	**0.003***	**0.35**
GFAP	13	6.70 (10.52)	36.38	48	0.00 (3.91)	29.54	242	0.218	0.16
Follow-up									
NFL	7	47.40 (58.90)	20.00	32	27.15 (16.73)	20.00	112	1.000	0.00
IL-6	7	2.64 (10.66)	15.00	31	4.09 (4.12)	20.52	77	0.236	0.19
GFAP	7	4.56 (11.20)	16.86	24	0.00 (0.00)	15.75	78	0.777	0.05
	Premanifest			**HC**			***Premanifest vs HC***		
	n	*Median (IQR)*	*Mean rank*	*n*	*Median (IQR)*	*Mean rank*	*U*	*p*	r_b
Baseline									
NFL	13	37.00 (18.80)	40.00	54	20.25 (15.90)	32.56	273	0.216	0.15
IL-6	13	2.54 (3.98)	34.38	54	1.84 (2.27)	33.91	346	0.937	0.01
GFAP	13	6.70 (10.52)	23.85	29	0.00 (7.78)	20.45	158	0.406	0.13
Follow-up									
NFL	7	47.40 (58.90)	22.86	29	18.20 (12.90)	17.45	71	0.223	0.20
IL-6	7	2.64 (10.66)	19.86	29	2.08 (1.55)	18.17	92	0.704	0.06
GFAP	7	4.56 (11.20)	9.43	10	0.00 (6.36)	8.70	32	0.769	0.07

Notes. DM1 = myotonic dystrophy type 1; HC = healthy controls; NFL = neurofilament light chain; IL-6 = interleukin-6; GFAP =glial fibrillary acidic protein. IQR = interquartile range; *U* = Mann-Whitney *U* test; *p* = *p*-value; r_b = rank-biserial correlation coefficient. *p < 0.05; **p < 0.001.

levels between Premanifest and Manifest DM1 patients when adjusted for age. Finally, when comparing Premanifest DM1 patients and HC (again after adjusting for age), no significant differences were found in any of the studied biomarkers, at either baseline or follow-up (see Table 3).

Finally, analyses of sex-related differences in biomarker levels revealed significant differences based on patient sex. After adjusting for age, male DM1 patients showed significantly higher baseline levels of both NFL and IL-6 compared to females, with moderate effect sizes (NFL, mean rank 41.00 vs 30.31; U= 425; p = 0.028*; rb= 0.26; IL-6, mean rank 41.12 vs 30.19; U= 421; p = 0.025*; rb= 0.27; see Table 4). At follow-up, the difference in NFL levels was not statistically significant; however, a moderate effect size indicated a continued trend toward higher NFL levels in males. No sex-differences were observed in the rest of the outcomes (CTG, MIRS, education, cognitive performance and structural brain outcomes) among DM1 patients (see Supplementary Table 2). Only, a moderate effect size was observed for WML, with men showing a higher burden of WML than women—although this difference did not reach statistical significance, likely due to the small sample size. Additionally, no sex-related differences in biomarker levels were observed among HC.

In contrast, analyses based on the sex of the disease-transmitting parent (i.e., inheritance pattern) showed no significant differences in biomarker levels between patients with maternal versus paternal inheritance after age adjustment ($p > 0.05$; $r < 0.15$, small effect sizes).

3.3. Longitudinal analyses of blood-based serum biomarkers

Analyses of potential selective attrition revealed no systematic group differences in DM1 patients, except for significantly higher baseline NFL levels and slightly lower attention scores among participants who discontinued the study.

Results of the intragroup and intergroup longitudinal analyses are provided in more detail in Supplementary Table 3 and Supplementary Table 4.

In intragroup longitudinal analyses, no statistically significant changes in biomarker levels were observed in either the DM1 patient group or the HC group. In contrast, within the Premanifest DM1 subgroup, a significant increase in IL-6 was detected at follow-up, with a large effect size ($p = 0.046$*; $r_b = 0.75$) (see Fig. 2). At baseline, Premanifest DM1 patients exhibited lower IL-6 levels compared to Manifest DM1 patients; however, following the observed increase, their IL-6 levels at follow-up reached a level comparable to that of the Manifest DM1 group.

Intergroup longitudinal analyses, adjusted for the time elapsed between assessments, revealed no significant differences in the rate of change (delta) in biomarker levels between DM1 patients and HC, indicating a similar progression over the four-year period.

3.4. Correlation analyses

Fig. 3 presents the results of Spearman's correlation analyses in DM1

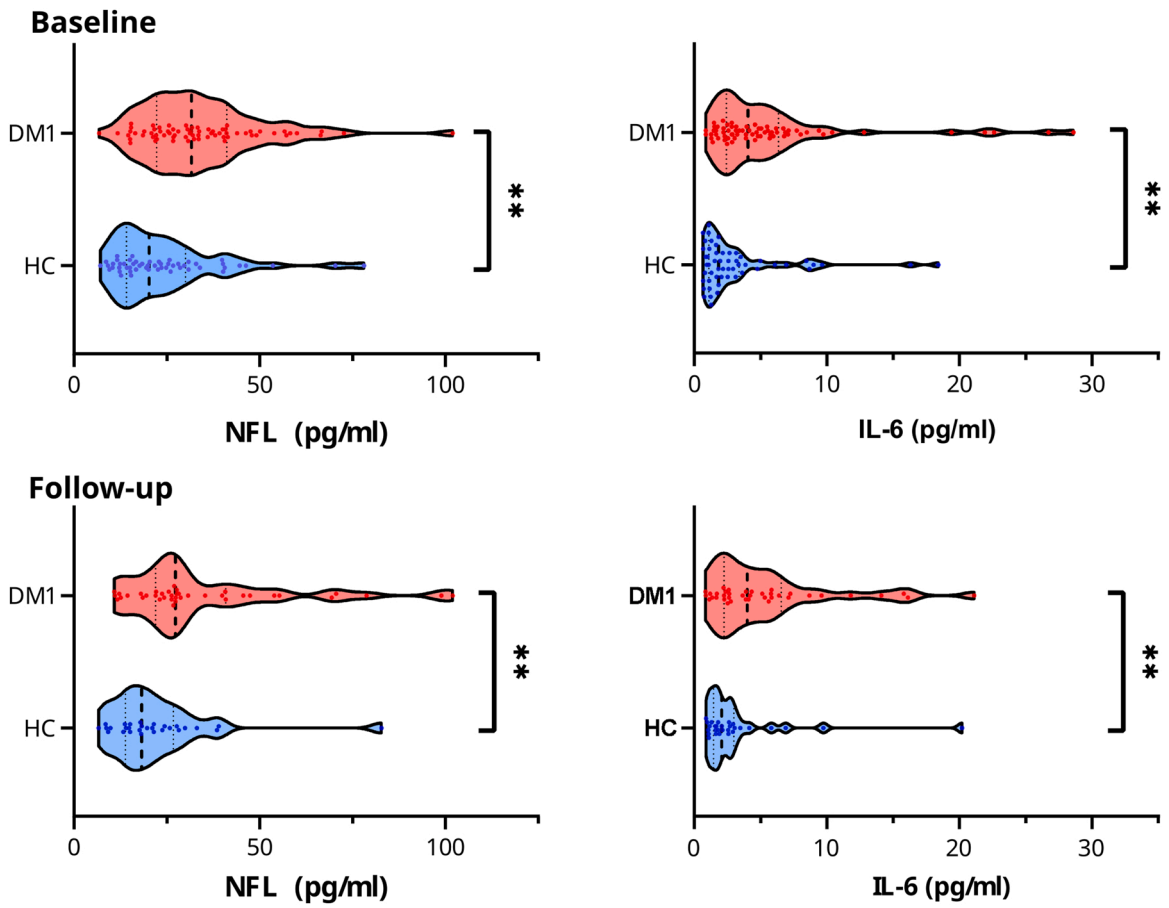

Fig. 1. Violin Plots illustrating Serum NFL and IL-6 Concentrations in patients with DM1 and HC. *Notes*. DM1 = myotonic dystrophy type 1; HC = healthy controls; NFL = neurofilament light chain; IL-6 = interleukin-6. Data represent cross-sectional biomarker measurements from the overall cohort of DM1 patientsand HC at each time point. *p < 0.05; **p < 0.001.

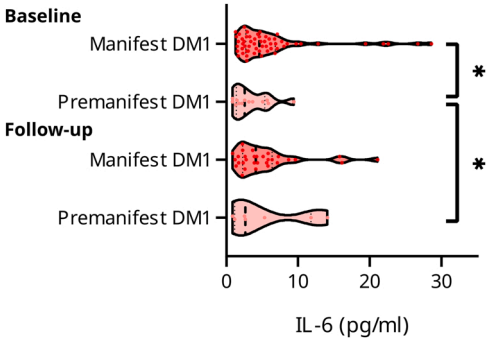

Fig. 2. Violin Plots illustrating Serum IL-6 Concentrations in Manifest DM1 and Premanifest DM1 patients at baseline and follow-up. *Notes.* DM1 = myotonic dystrophy type 1; IL-6 = interleukin-6. Baseline data include the full cohort of premanifest and manifest DM1 patients to illustrate cross-sectional differences. Follow-up data are shown only for participants with available longitudinal measurements. *p < 0.05; **p < 0.001.

patients, examining the associations between baseline levels of NFL, IL-6, GFAP, and various outcomes, including genetic, muscular, cognitive, and brain structural measures, after adjusting for age.

NFL levels correlated positively with the molecular defect (CTG repeat length), and negatively with cognitive performance, including estimated IQ, attention/processing speed, memory, and visuoconstruction domains. NFL levels also showed a significant negative correlation with GM volume, and a non-significant but moderate negative association with WML severity.

IL-6 levels correlated positively with both CTG repeat length and muscular impairment (MIRS), and negatively with cognitive performance across attention/processing speed, memory, visuoconstruction, and executive function domains. IL-6 also demonstrated non-significant but moderate correlations with reduced GM volume and increased WMLs. Finally, GFAP levels showed a significant negative correlation with muscular impairment (MIRS). Although not statistically significant, GFAP was also moderately associated with reduced WML burden.

Regarding the association between age and biomarker levels, baseline NFL and GFAP concentrations were positively correlated with age in DM1 patients, whereas in HC, all three baseline biomarkers (NFL, IL-6, GFAP) showed positive correlations with age (see Supplementary Figure 1). Regarding correlations between biomarkers, in DM1 patients, baseline NFL levels were positively associated with both baseline IL-6 levels and with follow-up NFL and GFAP levels. Baseline IL-6 correlated with follow-up IL-6 levels, while baseline GFAP was associated with follow-up GFAP and NFL levels. In HC, all biomarkers were positively correlated with each other and with age.

4. Discussion

This study explored serum biomarkers of neural damage (NFL, GFAP) and inflammation (IL-6) in DM1 patients, examining their cross-sectional and longitudinal profiles over a four-year period. Associations with muscular, molecular, cognitive, and structural brain outcomes were explored, along with potential sex-related differences. Several key findings emerged, supporting prior evidence while providing novel insights into the role and clinical utility of these biomarkers in DM1.

Table 4
Sex-related differences in serum NFL, IL-6, and GFAP levels among DM1 patients, adjusted for age.

	Male			Female			*Male vs Female*		
	n	*Median (IQR)*	*Mean rank*	*n*	*Median (IQR)*	*Mean rank*	*U*	*p*	r_b
Baseline									
NFL	34	34.85 (11.00)	41.00	36	24.95 (20.05)	30.31	425.00	**0.028***	**0.26**
IL-6	34	4.96 (6.19)	41.12	36	3.31 (3.86)	30.19	421.00	**0.025***	**0.27**
GFAP	28	0.00 (11.90)	26.68	33	0.00 (5.61)	34.67	341.00	0.080	0.22
Follow-up									
NFL	16	29.05 (23.10)	23.81	23	26.80 (21.90)	17.35	123.00	0.082	**0.28**
IL-6	15	2.76 (3.08)	17.87	23	5.13 (4.74)	20.57	148.00	0.464	0.12
GFAP	11	0.00 (0.00)	13.18	20	0.00 (5.21)	17.55	79.00	0.201	0.23

Notes. DM1 = myotonic dystrophy type 1; HC = healthy controls; NFL = neurofilament light chain; IL-6 = interleukin-6; GFAP =glial fibrillary acidic protein. IQR = interquartile range; *U* = Mann-Whitney *U* test; *p* = *p*-value; r_b = rank-biserial correlation coefficient. *p < 0.05.

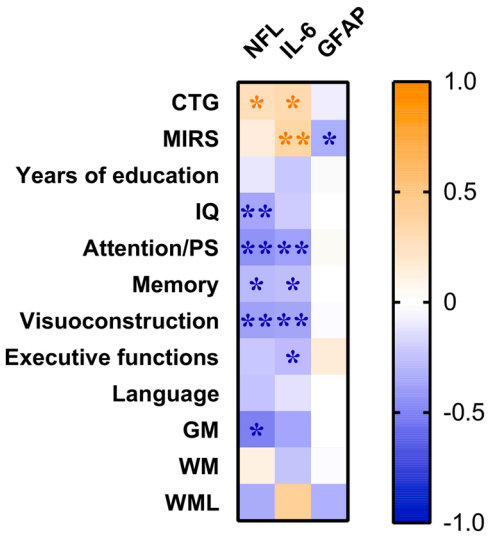

Fig. 3. Correlogram illustrating baseline biomarker levels, genetic, muscular, cognitive, and brain outcomes in DM1 patients. *Notes*. DM1 = myotonic dystrophy type 1; NFL = neurofilament light chain; IL-6 = interleukin-6; GFAP =glial fibrillary acidic protein; CTG = cytosine thymine guanine; MIRS = muscular impairment scale; IQ = intelligence quotient; PS = processing speed; GM = grey matter volume; WM = white matter volume; WML = white matter lesions. *$p < 0.05$; **$p < 0.001$.

DM1 patients exhibited significantly elevated levels of NFL and IL-6 compared to HC at both baseline and follow-up, indicating persistent neural damage and systemic inflammation. These findings are consistent with prior research reporting elevated neural damage and inflammation in DM1 (van der Plas et al., 2022; Laforce et al., 2022; Rossi and Silvestri, 2023; Saak et al., 2021; Azotla-Vilchis et al., 2021; Johansson et al., 2000; Mammarella et al., 2002). Interestingly, GFAP levels did not differ significantly between groups, suggesting that glial activation may be less prominent, transient, or less reliably detectable in blood in DM1.

It should be noted, however, in our dataset a substantial number of GFAP samples were below the assay's lower limit of detection, which may have limited our ability to identify potential group differences. A possible explanation is thatour biomarker measurements were obtained using the ELLA Automated Immunoassay System, whereas other studies employed the SIMOA platform (van der Plas et al., 2022; Laforce et al., 2022; Saak et al., 2021), which provides slightly higher analytical sensitivity. These methodological differences, together with variations in cohort size and composition, may partly account for the higher GFAP concentrations and detection rates reported in previous studies (van der Plas et al., 2022; Saak et al., 2021).

In Premanifest DM1 individuals, NFL and GFAP levels did not differ significantly from either manifest DM1 or HC. This contrasts with a previous study suggesting intermediate NFL levels between Manifest DM1 and HC, and elevated GFAP levels (van der Plas et al., 2022). Notably, IL-6 showed a dynamic pattern: while initially lower in Premanifest patients compared to Manifest DM1 patients at baseline, IL-6 levels significantly increased over the four-year follow-up, reaching levels comparable to those seen in Manifest DM1. This pattern suggests that inflammation may begin subtly and escalate gradually in DM1, supporting its relevance in the preclinical phase, which could be useful as a potential marker of phenoconversion in clinical trials. This observation may also indicate a link between IL-6 and muscular impairment in DM1, as muscular involvement is the defining feature distinguishing Premanifest from Manifest DM1 patients.

Regarding sex-related differences, male DM1 patients exhibited significantly increased NFL and IL-6 levels, while no significant differences were observed in GFAP levels. This contrasts with previous research reporting that NFL and GFAP levels were not predicted by sex in DM1 (van der Plas et al., 2022). One possible explanation is that males may experience greater disease severity in DM1 (Dogan et al., 2016), which could be reflected in higher levels of neuroaxonal damage and systemic inflammation, potentially influenced by sex-specific factors, such as hormonal or immune differences.

Elevated NFL in males likely reflects greater CNS vulnerability, consistent with the higher burden of WML observed in this and previous studies (Garmendia et al., 2024b). Interestingly, this biological vulnerability does not necessarily translate into global cognitive deficits, as overall cognitive performance and educational attainment were similar between sexes, suggesting potential resilience or compensatory mechanisms. Higher IL-6 levels in males may reflect increased systemic inflammation and overall disease severity, aligning with reports of worse clinical outcomes in male DM1 patients (Dogan et al., 2016). Further research is needed to clarify the mechanisms underlying sex-related differences in DM1, including potential biological vulnerabilities and protective factors contributing to cognitive resilience.

With respect to biomarker progression over time, no significant longitudinal changes were observed in NFL, IL-6, or GFAP levels. The observed rate of change was similar between DM1 patients and HC, consistent with earlier reports suggesting biomarker stability over similar timeframes (van der Plas et al., 2022). As previously noted, IL-6 was the exception, with a significant increase observed in Premanifest individuals. The overall biomarker stability may reflect the slow and heterogeneous nature of disease progression in DM1, highlighting the potential need for longer-term or more frequent follow-up intervals—especially in early or asymptomatic stages—to capture meaningful biological changes, or to identify biomarkers that are more sensitive in the short term.

In terms of biomarker associations, both NFL and IL-6 showed significant correlations with molecular, muscular, cognitive, and structural brain outcomes in DM1.

Both biomarkers were significantly associated with CTG repeat length, supporting the link between neural damage, inflammation, and the genetic basis of the disease. This aligns with previous findings showing that NFL levels can be predicted by the interaction between age

and estimated progenitor allele length (van der Plas et al., 2022). Increased inflammation (as measured by IL-6) was associated with greater muscular impairment, consistent with previous research linking RNA toxicity in DM1 to increased expression of inflammatory cytokines in skeletal muscle (Ozimski et al., 2021), further supporting a bidirectional relationship between muscular and inflammatory involvement. Importantly, while IL-6 was associated with muscular and cognitive outcomes, its exact origin cannot be determined. Skeletal muscle likely represents a major source, but systemic inflammation —from muscle pathology, metabolic disturbances, premature immunosenescence, and multisystemic involvement— may also contribute. Thus, IL-6 should be viewed as a marker of overall disease activity rather than a CNS-specific indicator. Interestingly, GFAP levels were paradoxically associated with less muscular impairment.

With respect to cognitive function, elevated levels of NFL and IL-6 were associated with poorer cognitive performance, reinforcing their potential as peripheral indicators of CNS dysfunction in DM1. Specifically, higher NFL levels were associated with lower IQ and deficits across multiple cognitive domains, while IL-6 was linked to reduced performance in nearly all assessed domains. In contrast, GFAP levels did not show any significant associations with cognitive outcomes.

In terms of structural brain outcomes, both NFL and IL-6 levels were associated with decreased GM volumes, although the IL-6 association did not reach statistical significance. IL-6 also demonstrated a moderate (but non-significant) association with increased WML burden, suggesting a possible role of inflammation in structural brain damage (Fornage et al., 2008). Indeed, previous findings have reported white matter disruption in DM1 (Labayru et al., 2022).

As expected, levels of neural damage markers (NFL and GFAP) were significantly associated with age in DM1, reflecting age-related increases in neural damage and glial activation. This trend is consistent with findings from the general population and other neurological conditions (Benkert et al., 2022), as well as previous work in DM1 (Nicoletti et al., 2022; Saak et al., 2021). In contrast, IL-6 levels did not correlate with age, suggesting that inflammatory processes in DM1 may be more closely tied to disease-specific mechanisms than to the normal aging process. This finding aligns with the concept of premature senescence in DM1 (Garmendia et al., 2024a; García-Puga et al., 2022; Hasuike et al., 2022; Mateos-Aierdi et al., 2015; Meinke et al., 2018), as elevated IL-6 is a key marker of immunosenescence— a state of chronic, low-grade inflammation associated with aging and implicated in various age-related diseases (Liu et al., 2023). This further supports the view of DM1 as a model of accelerated or premature aging.

This study has several limitations. First, the four-year follow-up period may be insufficient to capture meaningful longitudinal changes in biomarker levels, particularly given the slow and variable progression of DM1. Longer follow-up intervals or more frequent assessments may be necessary to capture subtle disease trajectories, especially in early or preclinical stages. Second, a substantial proportion of GFAP values were undetectable, which may reflect either true biological differences—such as a weaker glial response in DM1—or technical limitations in the sensitivity of the assay to detect low concentrations in peripheral blood. Third, as an inherent limitation of longitudinal studies, the follow-up cohort was smaller than the baseline cohort, which may introduce selection bias and limit generalizability. Although DM1 patients who discontinued had higher baseline NFL levels, group differences in NFL between DM1 and HC remained significant at follow-up, supporting the robustness of the cross-sectional findings. It remains uncertain, however, whether the intragroup change in NFL over time would have reached significance if all participants had remained. Despite this limitation, the longitudinal data provide valuable preliminary insights into biomarker trajectories. Finally, the relatively small neuroimaging sample may have limited the statistical power to detect associations between biomarkers and structural brain changes, highlighting the need for replication in larger cohorts.

5. Conclusions

This study provides valuable insights into the complex interplay between neural damage, inflammation, and clinical and CNS outcomes in DM1. Both processes are evident in affected individuals and appear to be linked to the genetic basis of the disease. Neural damage, in particular, was also associated with age, suggesting a potential link to aging-related mechanisms. Additionally, male patients demonstrated greater biomarker alterations.

NFL emerges as a promising biomarker of neural damage and cognitive impairment in DM1, supporting its utility as an indicator of CNS involvement in DM1. On the other hand, IL-6 may serve as a biomarker of systemic inflammation in DM1, with potential implications for muscular phenoconversion from Premanifest to Manifest and for cognitive impairment. However, their ability to track longitudinal disease progression remains uncertain. Future research should focus on larger, deeply phenotyped cohorts to define clinically meaningful thresholds and validate these biomarkers as predictors of disease trajectory and treatment response.

In conclusion, this study highlights the value of integrating blood-based biomarkers with clinical, cognitive, and neuroimaging data to improve our understanding of CNS and systemic involvement in DM1.

CRediT authorship contribution statement

Andone Sistiaga: Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Adolfo Lopez de Munain:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Laura Martins-Almeida:** Writing – review & editing, Investigation. **Ainhoa Alberro:** Writing – review & editing, Visualization, Investigation. **Pablo Iruzubieta:** Writing – review & editing, Methodology, Conceptualization. **David Otaegui:** Writing – review & editing, Methodology, Investigation. **Garazi Labayru:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Joana Garmendia:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization.

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Declaration of Competing Interest

All authors declare that they have no conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.brainresbull.2025.111688.

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