Supplementary Material

Serum neurofilament light chain and glial fibrillary acidic protein predicting multiple sclerosis after clinically isolated syndrome

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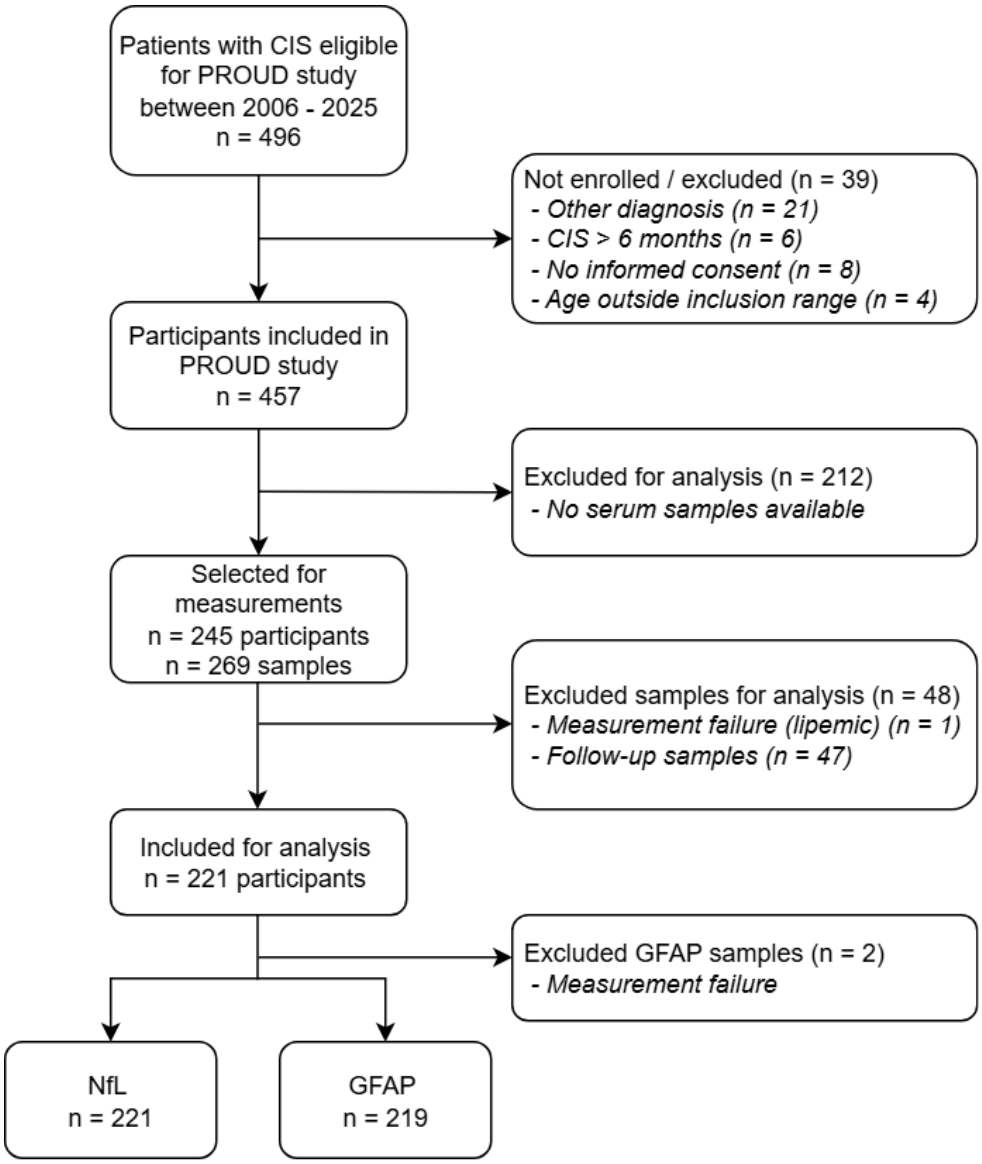

Figure S1 Flowchart of participant and sample selection

Table S1 | Clinical and demographic characteristics by NfL z-score tertiles				
	Low (≤1.41)	**Medium (1.41–2.20)**	**High (>2.20)**	**p-value**
N	74	74	73	
Sex (female, %)	49 (66.2)	50 (67.6)	51 (69.9)	0.89 [b]
Age at CIS (years)	34.4 [29.5, 42.5]	38.0 [29.4, 43.1]	30.0 [24.1, 35.3]	<0.001* [a]
Follow-up time (years)	5.1 [2.9, 7.8]	4.9 [2.6, 9.3]	5.3 [2.7, 8.0]	0.91 [a]
Time CIS-sampling (days)	96.0 [48.0, 153.0]	90.0 [25.8, 142.8]	78.0 [49.0, 127.0]	0.37 [a]
CIS topography				
Optic nerve	30 (40.5)	41 (55.4)	25 (34.2)	0.05 [b]
Brainstem	11 (14.9)	8 (10.8)	20 (27.4)	0.03* [b]
Cerebellar	3 (4.1)	1 (1.4)	7 (9.6)	0.07 [c]
Cerebral	7 (9.5)	6 (8.1)	10 (13.7)	0.53 [c]
Spinal cord	27 (36.5)	26 (35.1)	28 (38.4)	0.82 [b]
LP at baseline	58 (78.4)	54 (73.0)	53 (72.6)	
CSF IgG index	0.62 [0.54, 0.88]	0.63 [0.56, 0.92]	0.83 [0.60, 1.19]	0.08 [a]
CSF OCBs	38 (65.5)	35 (66.0)	40 (78.4)	0.27 [b]
MRI baseline				
≥9 T2 lesions	17 (23.0)	21 (28.4)	41 (56.2)	<0.001* [b]
≥1 GEL	11 (14.9)	21 (28.4)	34 (46.6)	<0.001* [b]
Infratentorial	19 (25.7)	19 (25.7)	40 (54.8)	<0.001* [b]
Spinal cord	30 (40.5)	29 (39.2)	35 (47.9)	0.18 [b]

Values are represented in n, % or median with interquartile range [IQR].
Abbreviations: CIS: clinically isolated syndrome. CSF: cerebrospinal fluid. GEL: gadolinium-enhancing lesion. IgG: immunoglobulin G. IQR: interquartile range. LP: lumbar puncture. MS: multiple sclerosis. NfL: neurofilament light chain. OCBs: oligoclonal bands.
* statistically significant with p-value <0.05
a = Kruskal-Wallis, b = Chi-squared test; c = Fisher's exact

Table S2 | Clinical and demographic characteristics by GFAP z-score tertiles				
	Low (≤-0.53)	**Medium (-0.53–0.53)**	**High (>0.53)**	**p-value**
N	73	73	73	
Sex (female, %)	54 (74.0)	49 (67.1)	46 (63.0)	0.36 [b]
Age at CIS (years)	32.3 [27.7, 41.8]	31.2 [24.7, 40.1]	33.6 [28.6, 39.6]	0.44 [a]
Follow-up time (years)	4.8 [2.9, 7.6]	5.0 [2.5, 7.7]	5.4 [2.6, 9.3]	0.78 [a]
Time CIS-sampling (days)	96.0 [38.0, 153.0]	90.0 [36.0, 142.0]	82.0 [49.0, 132.0]	0.85 [a]
CIS topography				
Optic nerve	35 (47.9)	29 (39.7)	30 (41.1)	0.36 b
Brainstem	10 (13.7)	10 (13.7)	18 (24.7)	0.11 [b]
Cerebellar	2 (2.7)	3 (4.1)	6 (8.2)	0.26 [c]
Cerebral	7 (9.6)	5 (6.8)	11 (15.1)	0.28 [c]
Spinal cord	23 (31.5)	30 (41.1)	28 (38.4)	0.38 [b]
LP at baseline	64 (87.7)	52 (71.2)	47 (64.4)	
CSF IgG index	0.63 [0.54, 1.18]	0.69 [0.56, 0.84]	0.69 [0.57, 1.11]	0.63 [a]
CSF OCBs	38 (60.3)	38 (76.0)	36 (76.6)	0.09 [b]
MRI baseline				
≥9 T2 lesions	23 (31.5)	26 (35.6)	30 (41.1)	0.44 [b]
≥1 GEL	13 (17.8)	24 (32.9)	29 (39.7)	0.01* [b]
Infratentorial	20 (27.4)	30 (41.1)	27 (37.0)	0.18 [b]
Spinal cord	23 (31.5)	34 (46.6)	36 (49.3)	0.002* [b]

Values are represented in n, % or median with interquartile range [IQR].
Abbreviations: CIS: clinically isolated syndrome. CSF: cerebrospinal fluid. GEL: gadolinium-enhancing lesion. GFAP: glial fibrillary acidic protein. IgG: immunoglobulin G. IQR: interquartile range. LP: lumbar puncture. MS: multiple sclerosis. OCBs: oligoclonal bands.
* statistically significant with p-value <0.05
a = Kruskal-Wallis, b = Chi-squared test; c = Fisher's exact

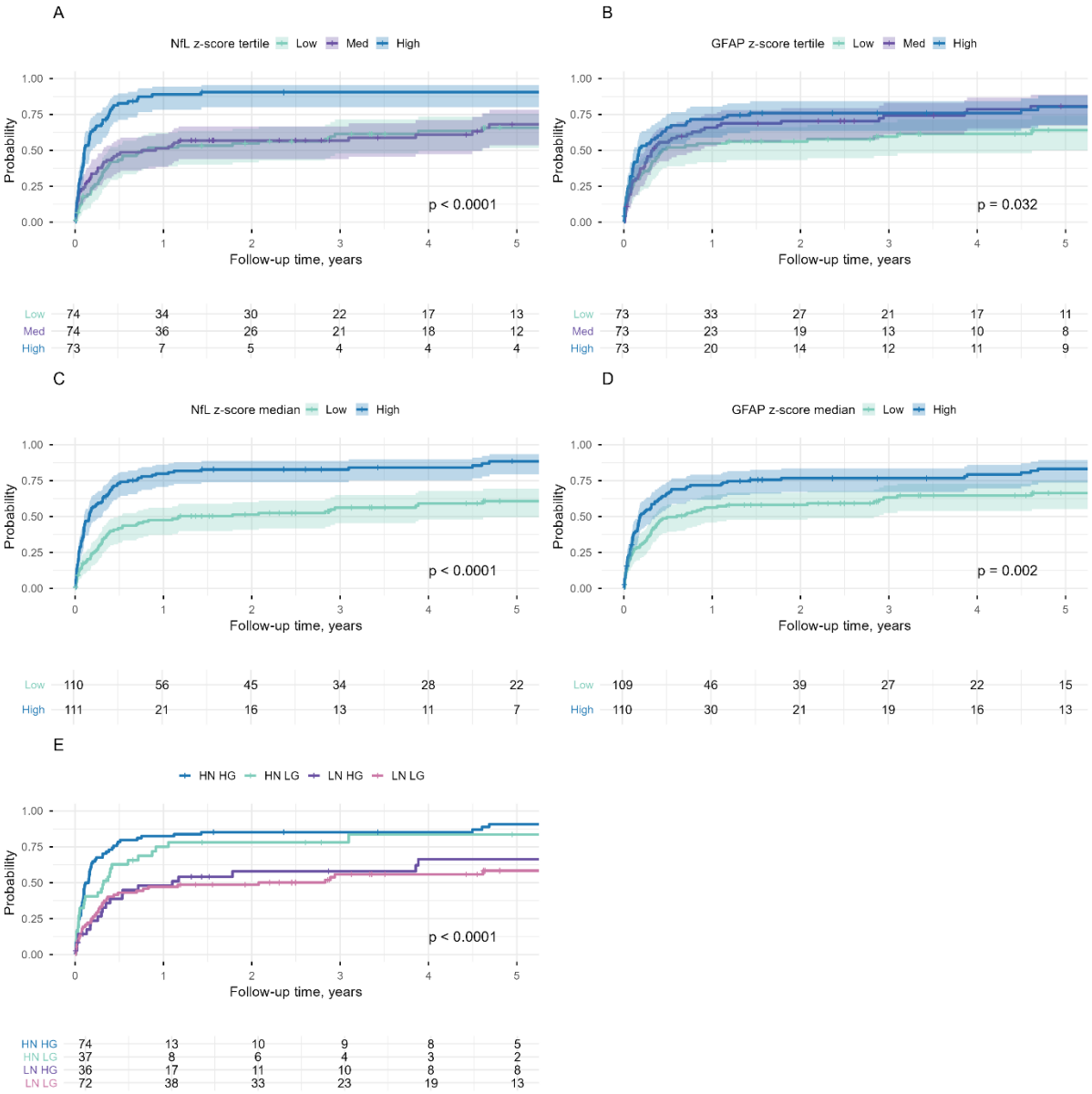

Figure S2 Zoomed-in Kaplan-Meier curves for time to McDonald 2024 MS diagnosis. Zoomed-in view of the Kaplan-Meier curves shown in Figure 2, displaying the identical survival curves with the x-axis restricted to the first 5 years of follow-up to highlight early divergence. Panel A shows the survival analyses of time to McDonald 2024 MS diagnosis by tertiles of NfL, with significant differences between High >2.20 (blue), Medium 1.41-2.20 (purple) and Low ≤1.41 (green). In panel B, time to MS diagnosis is shown for GFAP tertiles, with no significant differences by tertile subgroups (High >0.53 [blue], Medium -0.53-0.53 [purple], Low ≤-0.53 [green]). Panel C and D show the time to MS diagnosis, with NfL (C) divided by median into High ≥1.71 (blue) and Low <1.71 (green), and for GFAP (D) High ≥0.00 (blue) and Low <0.00 (green), with significant differences in both analyses between High and Low. Panel E shows a composite stratification of NfL and GFAP, with faster MS diagnoses in High NfL & High GFAP (blue) and High NfL & Low GFAP (green), compared to Low NfL & High GFAP (purple) and Low NfL & Low GFAP (pink).

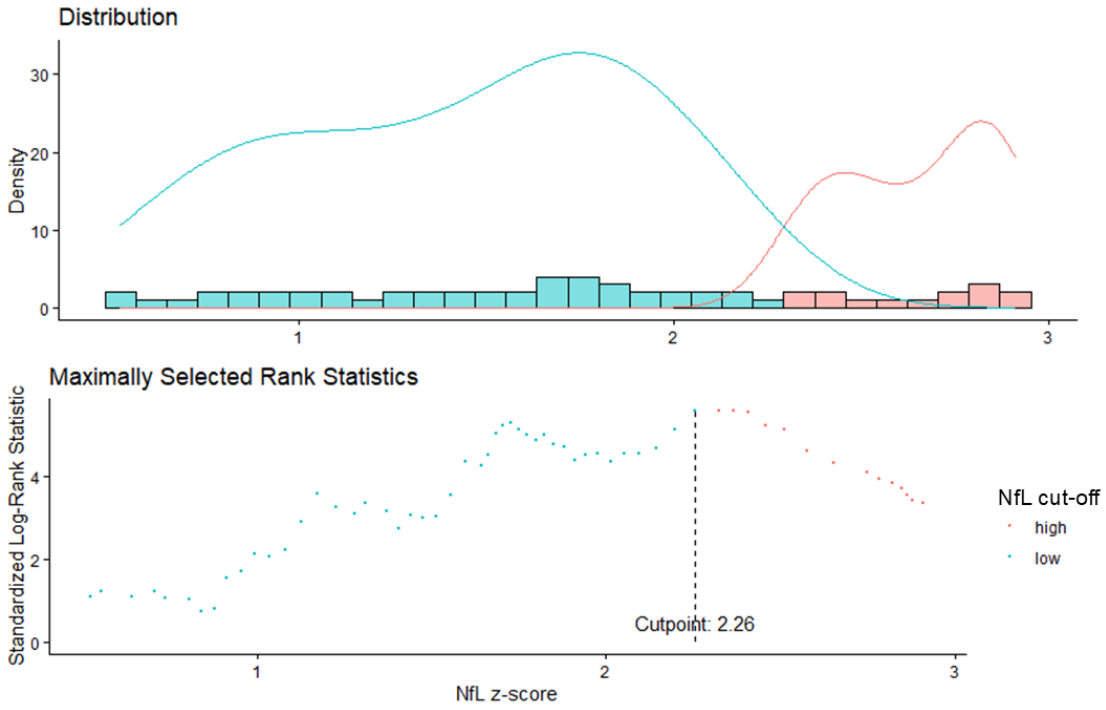

Figure S3 Determination of the optimal cut-off value for NfL z-scores. Using maximally selected rank statistics, the optical NfL z-score cut-off was calculation for our cohort. The vertical dashed line indicates the cut-off (z-score = 2.26) used to classify patients into low (n=159) and high (n=62) NfL groups.

Table S3 | Sensitivity analyses - Cox regression analyses for time to McDonald 2024 MS of MRI and NfL

Model	Variable	HR (95%CI)	p-value	C-index (SE)	LRT χ^2 (p-value)
MRI-1	MRI ≥9 T2	3.34 (2.41 – 4.63)	<0.001*	0.672 (0.02)	
MRI-1+NfL	MRI ≥9 T2	2.82 (1.99 – 3.98)	<0.001*	0.695 (0.02)	9.64 (0.002*) [a]
	NfL z-score	1.36 (1.12 – 1.66)	0.002*		
MRI-2	MRI ≥9 T2	3.15 (2.18 – 4.56)	<0.001*	0.714 (0.02)	
	MRI ≥1 GEL(s)	2.71 (1.88 – 3.91)	<0.001*		
MRI-2+NfL	MRI ≥9 T2	2.90 (1.98 – 4.24)	<0.001*	0.720 (0.02)	3.83 (<0.05*) [b]
	MRI ≥1 GEL(s)	2.52 (1.74 – 3.67)	<0.001*		
	NfL z-score	1.25 (1.00 – 1.56)	<0.05*		

Analyses were performed with multivariate Cox proportional hazards models, adjusted for sex and age at CIS. Only complete cases were used, with population in analyses of MRI-1/MRI-1+NfL n=217 and analyses of MRI-2/MRI-2+NfL factors n=195. Additive value of NfL was tested using Likelihood Ratio Test (LTR). Model comparisons: a = MRI-1 vs. MRI-1+NfL. b = MRI-2 vs. MRI-2+NfL.

Abbreviations. CI: confidence interval. CIS: clinically isolated syndrome. GEL: gadolinium-enhancing lesion. HR: hazard ratio. LRT: likelihood ratio test. NfL: neurofilament light chain. SE: standard error.

* statistically significant with p-value <0.05

Table S4 | Additional Cox regression analyses for time to McDonald 2024 MS of NfL, GFAP and risk factors

Model	Variable	HR (95%CI)	p-value	C-index (SE)	LRT χ^2 (p-value)
NfL, GFAP and risk factors					
NfL+wGRS	NfL z-score	1.34 (1.08 – 1.67)	0.01*	0.691 (0.02)	0.30 (0.58) [a]
	wGRS	1.05 (0.89 – 1.23)	0.58		
GFAP+wGRS	GFAP z-score	1.12 (1.02 – 1.21)	0.02*	0.680 (0.02)	0.10 (0.75) [b]
	wGRS	1.03 (0.87 – 1.21)	0.75		
NfL+GFAP+wGRS	NfL z-score	1.27 (1.00 – 1.60)	<0.05	0.690 (0.02)	1.77 (0.18) [c]
	GFAP z-score	1.08 (0.97 – 1.20)	0.17		
	wGRS	1.01 (0.87 – 1.20)	0.80		
NfL+EBV	NfL z-score	1.35 (1.09 – 1.68)	0.01*	0.689 (0.02)	1.30 (0.25) [d]
	Anti-EBNA1 IgG	1.06 (0.96 – 1.18)	0.26		
GFAP+EBV	GFAP z-score	1.13 (1.03 – 1.24)	0.01*	0.679 (0.02)	1.38 (0.24) [e]
	Anti-EBNA1 IgG	1.06 (0.96 – 1.18)	0.25		
NfL+GFAP+EBV	NfL z-score	1.27 (1.01 – 1.60)	0.04*	0.686 (0.02)	2.15 (0.14) [f]
	GFAP z-score	1.08 (0.98 – 1.21)	0.13		
	Anti-EBNA1 IgG	1.07 (0.96 – 1.19)	0.24		
NfL+HLA+EBV	NfL z-score	1.34 (1.07 – 1.66)	0.01*	0.694 (0.02)	0.58 (0.45) [g]
	HLA-DRB1*15:01	1.41 (0.99 – 2.02)	0.06		
	Anti-EBNA1 IgG	1.04 (0.94 – 1.16)	0.45		
GFAP+HLA+EBV	GFAP z-score	1.11 (1.02 – 1.23)	0.03*	0.683 (0.02)	0.70 (0.40) [h]
	HLA-DRB1*15:01	1.28 (0.96 – 1.97)	0.08		
	Anti-EBNA1 IgG	1.05 (0.94 – 1.16)	0.41		

Analyses were performed with multivariate Cox proportional hazards models, adjusted for sex, age and ≥9 T2 lesions on baseline MRI. Anti-EBNA1 IgG titres are log-transformed. Only complete cases were used, with population in analyses of NfL+GFAP n=217 and analyses of NfL+GFAP+risk factors n=189.

Additive value of combined biomarkers was tested using Likelihood Ratio Test (LTR). Model comparisons: a = NfL vs. NfL+wGRS, b = GFAP vs. GFAP+wGRS, c = NfL+wGRS vs. NfL+GFAP+wGRS, d = NfL vs. NfL+EBV, e = GFAP vs. GFAP+EBV, f = NfL+EBV vs. NfL+GFAP+EBV, g = NfL+HLA vs. NfL+HLA+EBV and h = GFAP+HLA vs. GFAP+HLA+EBV.

Abbreviations. CI: confidence interval. CIS: clinically isolated syndrome. EBNA1: Epstein Barr virus nuclear antigen-1. EBV: Epstein Barr virus. GFAP: glial fibrillary acidic protein. HLA: human leukocyte antigen. HR: hazard ratio. IgG: immunoglobulin G. LRT: likelihood ratio test. NfL: neurofilament light chain. Ref: reference. SE: standard error. wGRS: weighted genetic risk score.

* statistically significant with p-value <0.05