Quantifying synaptic dysfunction in multiple sclerosis by means of a cerebrospinal fluid biomarker panel: a pilot study

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INTRODUCTION

Emerging evidence highlights the role of **synaptopathy -** the dysfunction or loss of synapses - in multiple sclerosis (MS) pathophysiology (1).
Synaptic alterations can potentially result from both focal inflammation (e.g., active lesions) and diffuse chronic immune activation and neuronal loss (2).
Synaptic damage may underlie network disconnection and several clinical manifestations of MS, including **cognitive impairment** (1).
Recent technological advances have enabled the detection of synaptic proteins in cerebrospinal fluid (CSF) and blood, offering new opportunities to explore biomarkers of synaptopathy in neurological diseases (3).
Synaptic biomarkers have been thoroughly investigated in neurodegenerative diseases, but only few studies have been done in people living with MS (pwMS) (4).

OBJECTIVES

- **To test a large panel of CSF biomarkers of synaptic injury in pwMS**
- **To verify the association between CSF biomarkers of synaptic injury and clinical characteristics of MS**

METHODS

- People living with MS (**n: 105**) and with other neurological diseases (OND) (controls, **n: 30**) were retrospectively enrolled at the Section of Neurology, University Hospital of Perugia (Italy) with CSF samples collected at the time of diagnosis (drug-naïve) (**Tables 1 and 2**).
- All pwMS underwent CSF analysis and a complete clinical characterization as part of the routine diagnostic work-up for MS.
- CSF samples were analyzed through liquid chromatography/mass spectrometry (LC/MS) at the Institute of Neuroscience and Psychiatry, University of Gothenburg (Sweden) (**Table 3**).

Table 1 . Characteristics of the MS group

	RIS (n: 5)	RMS (n: 92)	PMS (n: 8)
Clinical characteristics			
Disease duration (months); median (IQR)	-	2 (5)	30 (63)
EDSS; mean ± SD	0	2.2 ± 1	3.4 ± 1.4
Relapses last year preceding CSF samplint yes-no; n (%)	0	88 (95.7)	2 (25)
Gd+ lesions on brain MRI; n (%)	0	62 (67.4)	1 (12.5)
CSF IgG OCB; n (%)	2 (40)	79 (85.9)	6 (75)

Legend. *CSF: cerebrospinal fluid. EDSS: Expanded Disability Status Scale. Gd+: gadolinium enhancing. IgG: immunoglobulin G. IQR: interquartile range. MRI: magnetic resonance imaging. MS: multiple sclerosis. n.s.: not significant. OCB: oligoclonal bands. PMS: progressive multiple sclerosis. RIS: radiologically isolated syndrome. RMS: relapsing multiple sclerosis. SD: standard deviation.*

PRELIMINARY RESULTS

- MS and OND were age- and sex-matched (**Table 2**).
- **CSF synaptic proteins were downregulated in MS vs OND** (**Figures 1 and 2**).
- A trend towards negative correlation with disease duration and EDSS (**Figure 3**) was found for most of the measured markers.

No significant differences were found in CSF synaptic markers between pwMS with and without relapses in the year preceding CSF sampling and with and without Gd+ lesions on brain MRI.

Figure 1. Volcano plot of CSF biomarkers (MS vs. OND). In red biomarkers with a –log10 (p-value) ≥ 2 are represented.

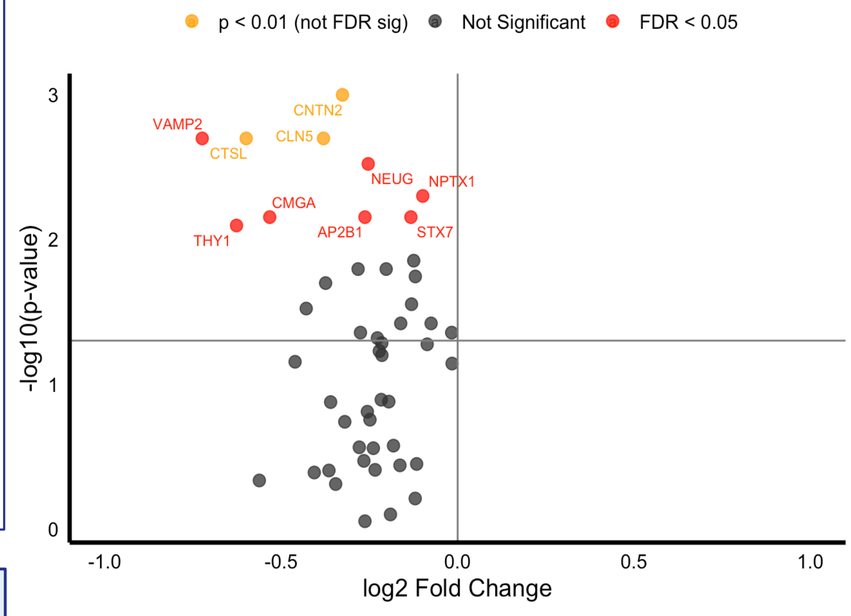


Table 2. Characteristics of the cohort

	MS (n: 105)	OND (n: 30)	p-value
Demographical features			
Age (years); mean ± SD	37.1 ± 12.1	40.3 ± 12.7	n.s.
Sex (F/M)	67/38	24/6	n.s.
MS clinical phenotypes			
RIS; n (%)	5 (4.8)	-	-
RMS; n (%)	92 (87.6)	-	-
PMS; n (%)	8 (7.6)	-	-

Legend. *MS: multiple sclerosis. n.s.: not significant. OND: other neurological diseases. PMS: progressive multiple sclerosis. RIS: radiologically isolated syndrome. RMS: relapsing multiple sclerosis. SD: standard deviation.*

Figure 2. Boxplot of most significantly changed CSF biomarkers (MS vs. OND).

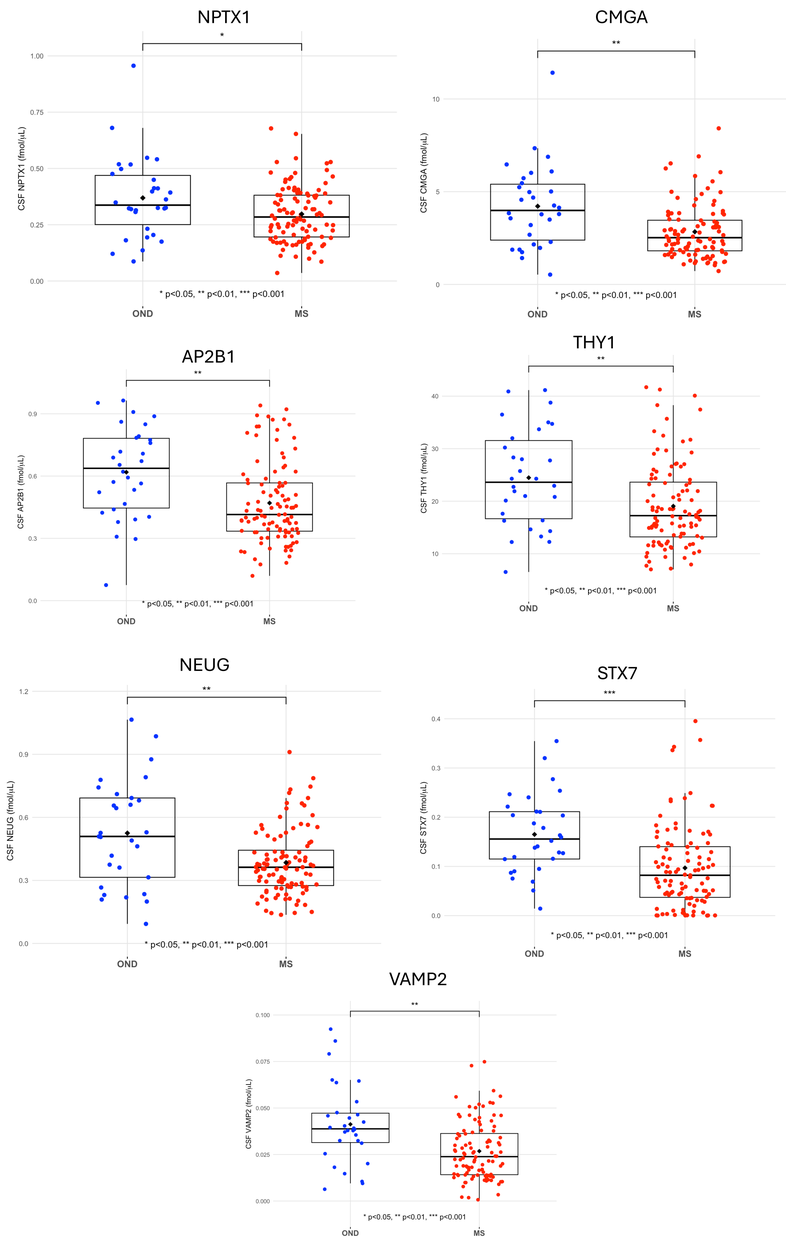
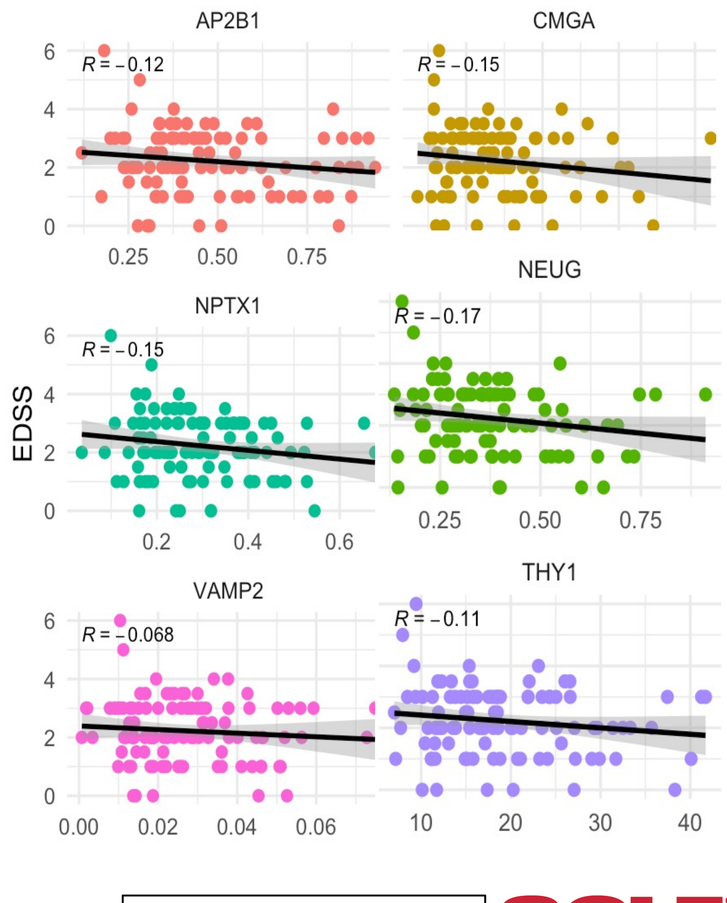


Table 3. List of the tested proteins

Protein name	Uniprot Entry name	Uniprot ID	Protein	Gene
1433Z	1433Z_HUMAN	P63104	14-3-3 protein zeta/delta	YWHAZ
A4	A4_HUMAN	P05067	Amyloid-beta precursor protein	APP
AGRB3	AGRB3_HUMAN	O60242	Adhesion G protein-coupled receptor B3	ADGRB3
AP2B1	AP2B1_HUMAN	P63010	AP-2 complex subunit beta	AP2B1
CMGA	CMGA_HUMAN	P10645	Chromogranin-A	CHGA
CNTN1	CNTN1_HUMAN	Q12860	Contactin-1	CNTN1
CNTN2	CNTN2_HUMAN	Q02246	Contactin-2	CNTN2
CPLX1	CPLX1_HUMAN	O14810	Complexin-1	CPLX1
CPLX2	CPLX2_HUMAN	Q6PUV4	Complexin-2	CPLX2
CSTN1	CSTN1_HUMAN	O94985	Calsyntenin-1	CLSTN1
GRIA4	GRIA4_HUMAN	P48058	Glutamate receptor 4	GRIA4
KCC2A	KCC2A_HUMAN	Q9UQM7	Calcium/calmodulin-dependent protein kinase type II subunit alpha	CAMK2A
NCAM2	NCAM2_HUMAN	O15394	Neural cell adhesion molecule 2	NCAM2
NEUG	NEUG_HUMAN	Q92686	Neurogranin	NRGN
NPTX1	NPTX1_HUMAN	Q15818	Neuronal pentraxin-1	NPTX1
NPTX2	NPTX2_HUMAN	P47972	Neuronal pentraxin-2	NPTX2
PEBP-1	PEBP-1_HUMAN	P30086	Phosphatidylethanolamine-binding protein 1	PEBP1
SCG2	SCG2_HUMAN	P13521	Secretogranin-2	SCG2
SEM3C	SEM3C_HUMAN	Q99985	Semaphorin-3C	SEMA3C
SEM6A	SEM6A_HUMAN	Q9H2E6	Semaphorin-6A	SEMA6A
SORL	SORL_HUMAN	Q92673	Sortilin-related receptor	SORL1
SORT	SORT_HUMAN	Q99523	Sortilin	SORT1
STX7	STX7_HUMAN	O15400	Syntaxin-7	STX7
SYUB	SYUB_HUMAN	Q16143	Beta-synuclein	SNCB
THY1	THY1_HUMAN	P04216	Thy-1 membrane glycoprotein	THY1
VAMP2	VAMP2_HUMAN	P63027	Vesicle-associated membrane protein 2	VAMP2
VGF	VGF_HUMAN	O15240	Neurosecretory protein VGF	VGF

Figure 3. Scatter plot showing a trend toward a negative correlation between synaptic protein levels and EDSS in pwMS



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CONCLUSIONS

- Our study shows that **CSF synaptic markers are decreased in pwMS** compared to controls.
- In this exploratory cohort, **no significant associations** were found between synaptic markers and demographic or clinical characteristics of the disease. A trend towards an negative correlation with disease duration and EDSS has been observed.
- These preliminary results suggest that CSF synaptic markers in MS show a trend that is different from that observed in primary neurodegenerative disorders, where they have consistently been found increased.
- It could be hypothesized that, in MS, CSF concentrations of synaptic proteins reflect the **combined result of both acute synaptic damage** and **synaptic engulfment** by microglial cells, eventually leading to a decrease in synaptic connections and network failure.
- **Further investigations on larger cohorts and multiple test correction are deemed to validate these preliminary results.**

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QUESTIONS?

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