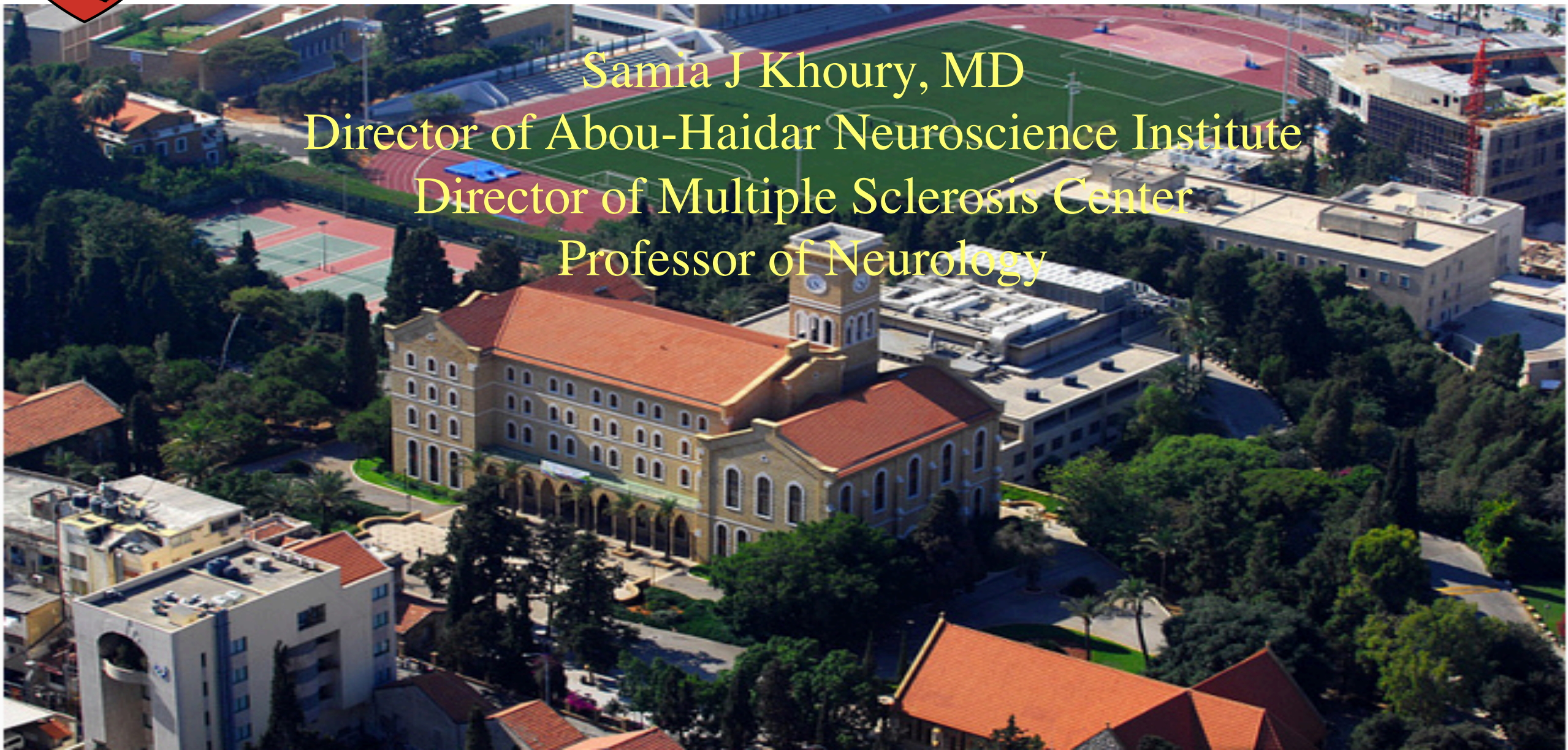
Biomarkers For MS



Samia J Khoury, MD
Director of Abou-Haidar Neuroscience Institute
Director of Multiple Sclerosis Center
Professor of Neurology

Disclosures

None

Learning Objectives

Process of biomarker development

Biomarkers in clinical practice

Serum NfL as potential biomarkers

Key Messages

Serum NfL levels are elevated in MS patients

Serum NfL is not specific to MS and it increases gradually with normal ageing

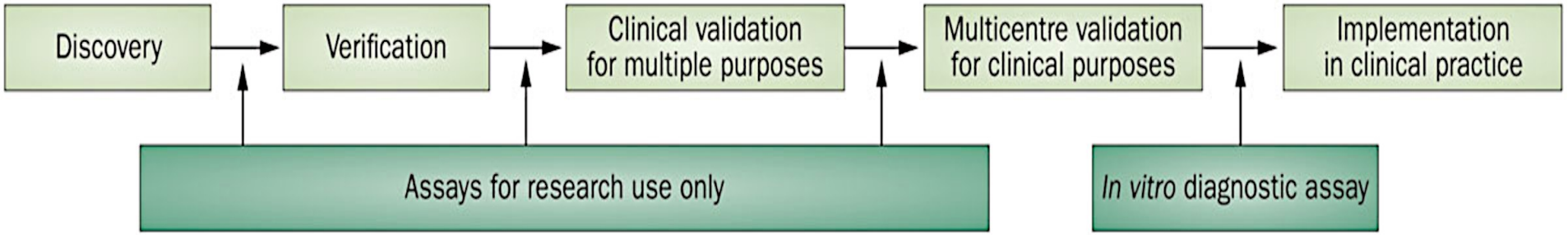
Levels correlate with number of T2 and GD+ lesions on MRI

Baseline sNfL can predict Disability progression and change in brain volume after 5 –ears or more

Characteristics of an Ideal Biologic Marker

- Measurable in a body fluid that is easy to obtain
- Easily measurable with routine /affordable laboratory procedures
- Involved in the disease pathogenesis
- Correlated with clinical disease activity and disability
- High sensitivity in detecting relevant disease activity
- High specificity (not influenced by other diseases, complications such as infection)
- Correlated with radiological disease activity markers, such as MRI
- Undergo rapid normalization under therapy in responders
- Undergo no normalization under therapy in non-responders

Schematic Representation Of The Process Of Biomarker Development



Nature Reviews | Neurology

Types Of Biomarker Needed In MS

- Supporting diagnosis
- Identify converters from CIS to MS
- Identify converters from RR to SPMS
- Predicting disease severity
- Predicting response to treatment
- Predicting risk of adverse events

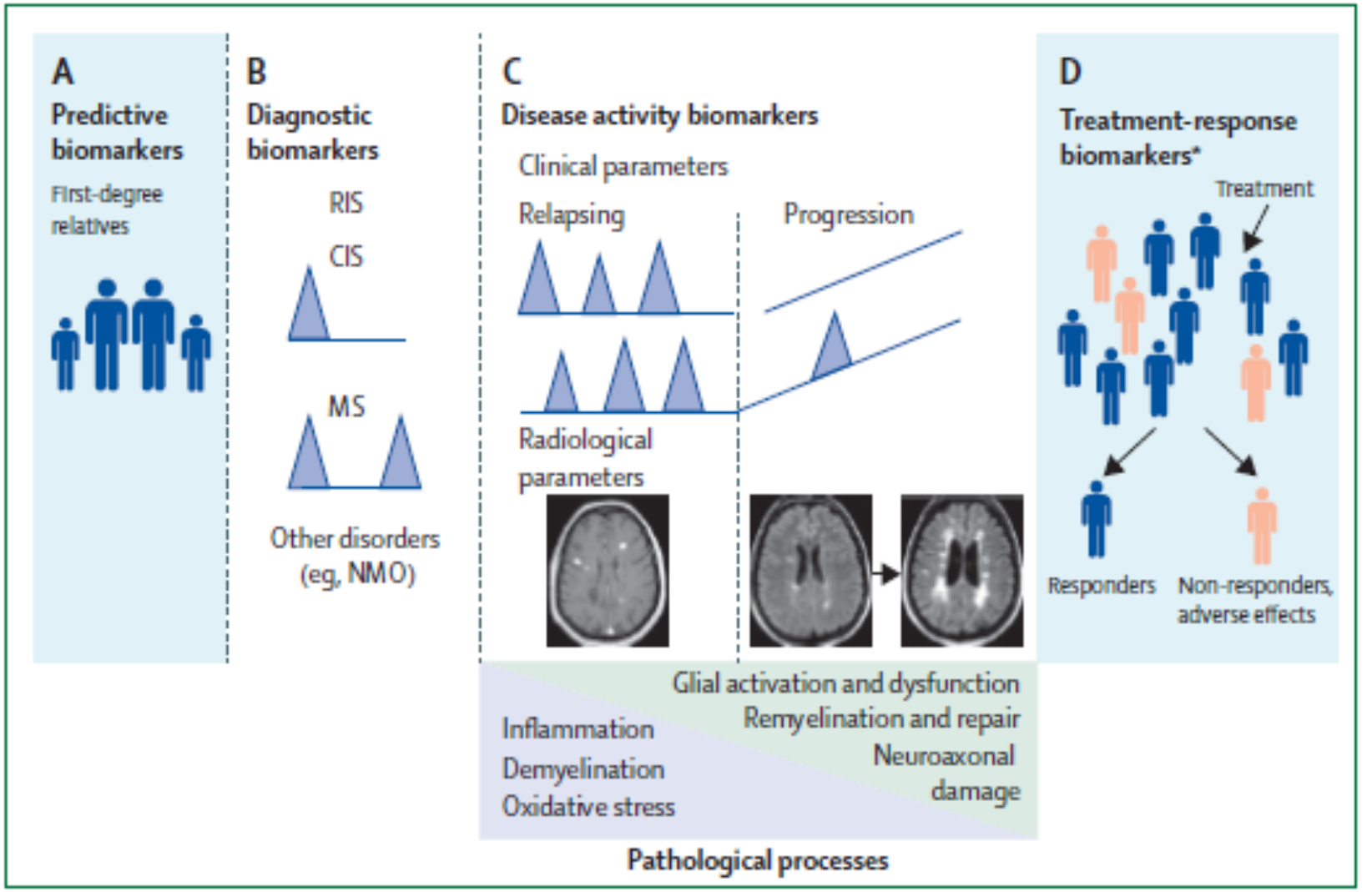


Figure 1: Types of biomakers in MS

Manuel Comabella, Xavier Montalban. Lancet Neurol 2014; 13: 113–26

	Category	Body fluid	Biomarker test	Target population	Prevalence	Effect on clinical decision making?
Anti-natalizumab antibodies	Natalizumab-response biomarker for an adverse effect	Serum	ELISA	Patients with MS given natalizumab	4·1–6·1% of natalizumab-treated patients[94,95]	Yes; if persistently positive, treatment with natalizumab should be discontinued[119]
Neutralising antibodies	Interferon-beta-response biomarker	Serum*, PBMCs	CPE*, luciferase gene-reporter assay*, MxA-based assays	Patients with MS given interferon beta	2–45% of patients given interferon beta†	Yes; if present, a switch to a non-interferon-beta treatment should be considered[102]
IgG oligoclonal bands	Diagnostic	CSF, serum	IEF/IB	Patients suspected to have demyelinating disease	>95% of patients with MS[104]	No
IgG index	Diagnostic	CSF, serum	Formula‡	Patients suspected to have demyelinating disease	Increased in 70% of patients with MS[104]	No
Anti-aquaporin-4 antibodies	Diagnostic	Serum*, CSF	IHC*, CBA*, ELISA*, RIPA, FCMA, FIPA, WB	Patients with clinical and MRI features suggestive of neuromyelitis optica or neuromyelitis optica spectrum disorders	Almost 75–90% of patients with neuromyelitis optica; almost absent in MS patients[105]	Yes; if present, diagnosis should be separate from typical MS[120]
Anti-JC virus antibodies	Natalizumab-response biomarker for an adverse effect	Serum*, plasma	ELISA	Patients with MS eligible for natalizumab or those receiving natalizumab	50–60% of patients with MS[121]	Yes; test result allows estimation of the patient's risk for progressive multifocal leukoencephalopathy[108]
Anti-VZV antibodies	Fingolimod-response biomarker for an adverse effect	Serum*, plasma	ELISA	Patients with MS eligible for fingolimod	90–95% of patients with MS[122]	Yes; seronegative patients should be vaccinated at least 1 month before start of fingolimod[105,123]

ELISA=enzyme-linked immunosorbent assay. MS=multiple sclerosis. PBMCs=peripheral blood mononuclear cells. CPE=cytopathic effect assay. MxA=myxovirus resistance protein A. IEF/IB=isoelectric focusing combined with immunoblotting. IHC=indirect immunohistochemistry. CBA=cell-based assays. RIPA=radioimmunoprecipitation assay. FCMA=flow cytometric assay. FIPA=fluoroimmunoprecipitation assay. WB=western blotting. VZV=varicella zoster virus. *Most commonly used for biomarker detection. †Percentages are dependent on the interferon-beta formulation and dose. ‡Formula=(CSF/serum IgG)/(CSF/serum albumin).

Table 2: **MS biomarkers in use in clinical practice**

Manuel Comabella, Xavier Montalban. Lancet Neurol 2014; 13: 113–26

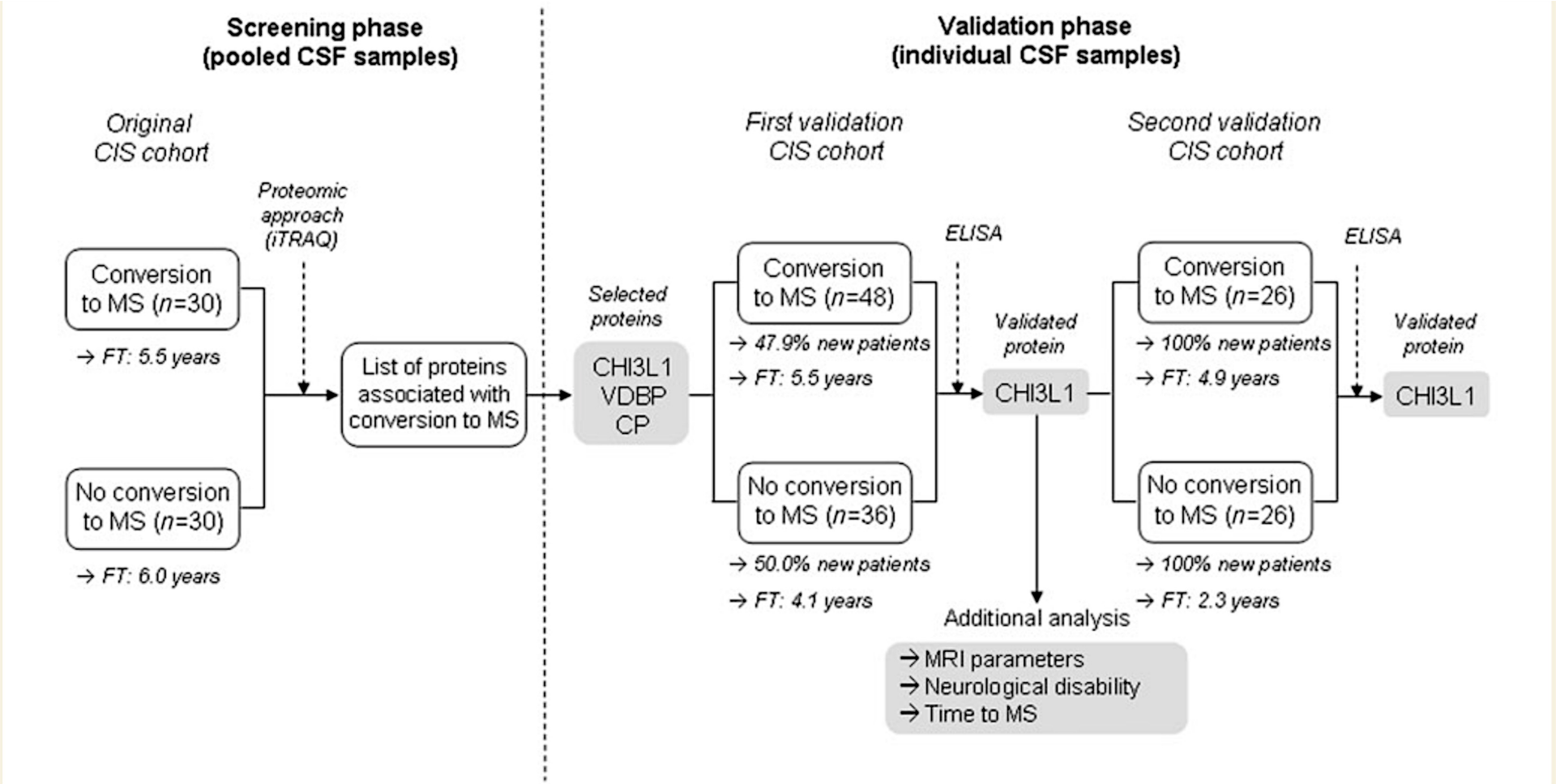
Potential Biomarkers That Have Shown Some Replication Across Studies

- Neurofilament light in CSF and serum
- MOG antibodies
- Chitinase 3-like 1 and Chitinase 3-like 2
- Certain miRNAs
- Low affinity serum antibody microarrays

Chitinase 3-like 1

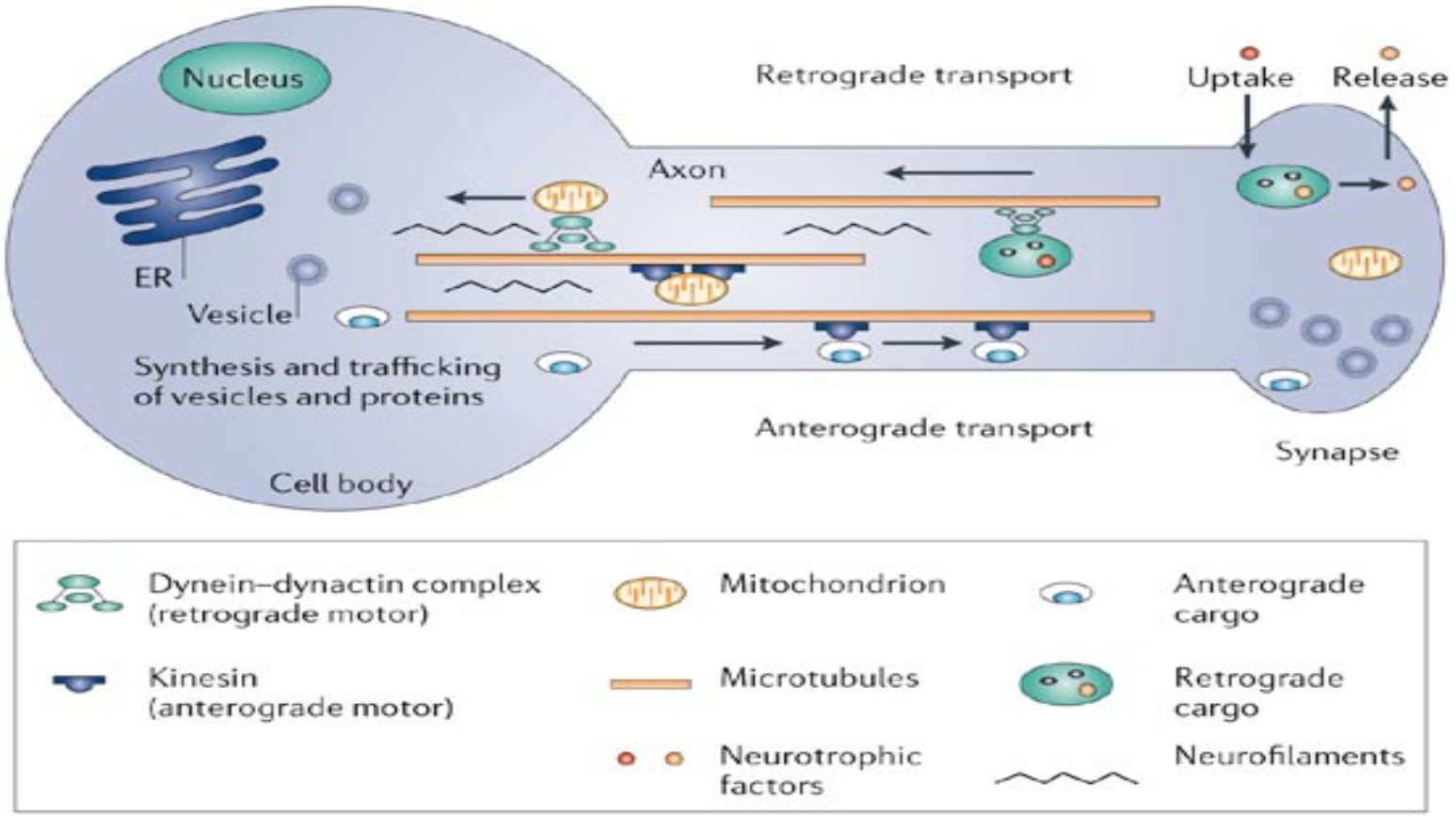
- CH3L1 is a member of the family of chitinases and chitinase-like proteins containing a highly conserved glyco-18 domain as common feature. For these proteins, chitin is the only documented substrate
- CHI3L1 can bind chitin but lacks chitinolytic activity
- In the CNS, CHI3L1 expression has been mainly observed in astrocytes of monkeys and humans with lentiviral encephalitis, and patients with brain infarcts

CSF Chitinase 3-like 1 Levels Are Associated With Conversion To MS

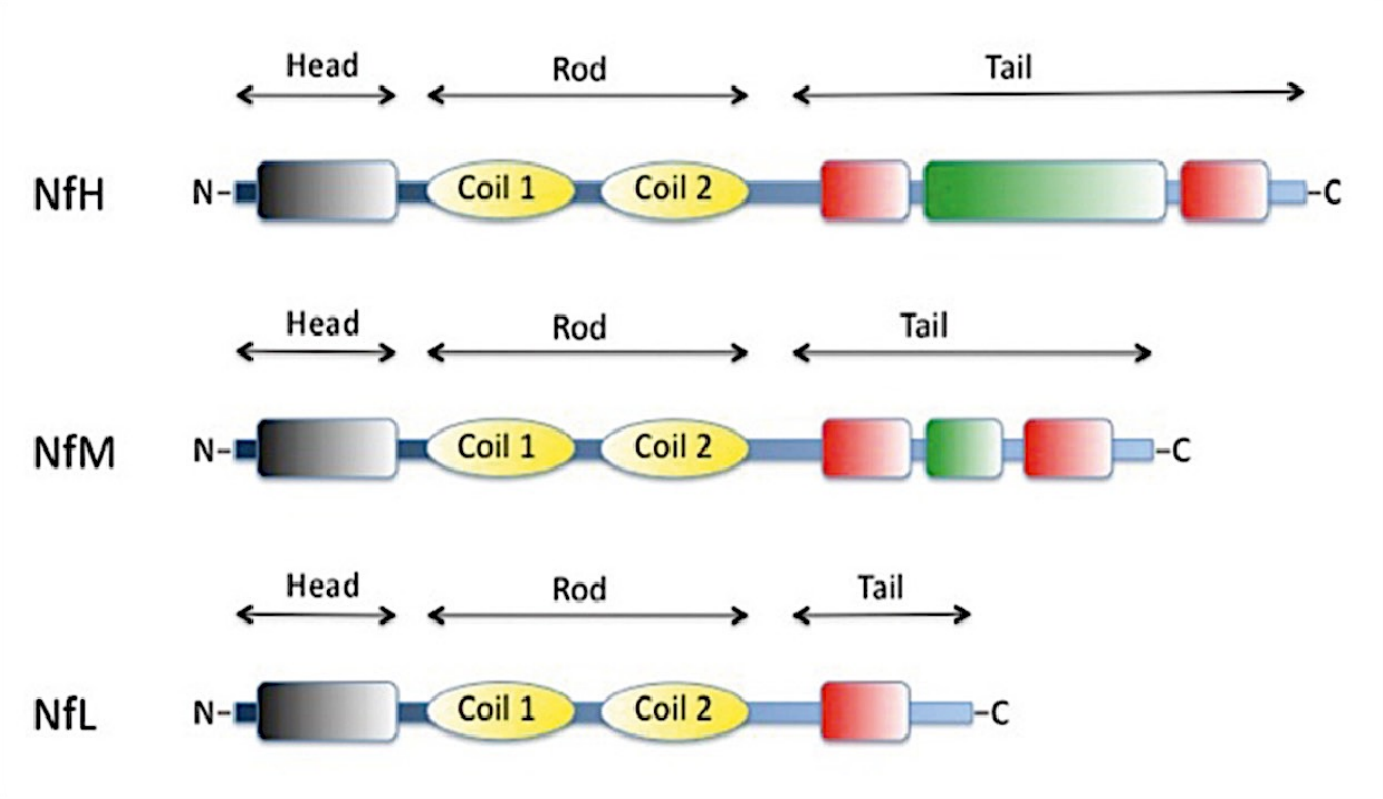


Brain 2010: 133; 1082–1093

Neurofilaments (NF)



Neurofilaments (NF)



NfH = neurofilament heavy chain; NfL = neurofilament light chain; NfM = neurofilament intermediate chain; N = N-terminus; C = C-terminus.

- Neurofilaments could be used in clinical practice as surrogate endpoints of neuroaxonal damage

- CSF concentrations of both NF-L and NF-H have been noted to be increased in patients with MS:
    - during relapse
    - related to radiological activity
    - related to disability and conversion to SPMS in 14 year follow up studies
    - noted to have prognostic value for conversion to MS in CIS patients-1yr

- NF-L seems to be a more accurate sign of acute axonal damage associated with inflammation than does NF-H

- NF-H better captures chronic axonal damage and shows a stronger association with disability progression

- A reduction in CSF NF-L is associated with MS treatments such as natalizumab, mitoxantrone, and rituximab, and NF-L has been proposed as a surrogate endpoint for treatment efficacy

Measurement of sNFl

CSF NfL is easily measured by ELISA, but serum Nfl are 70-100 X lower than CSF

How do we measure sNfL:

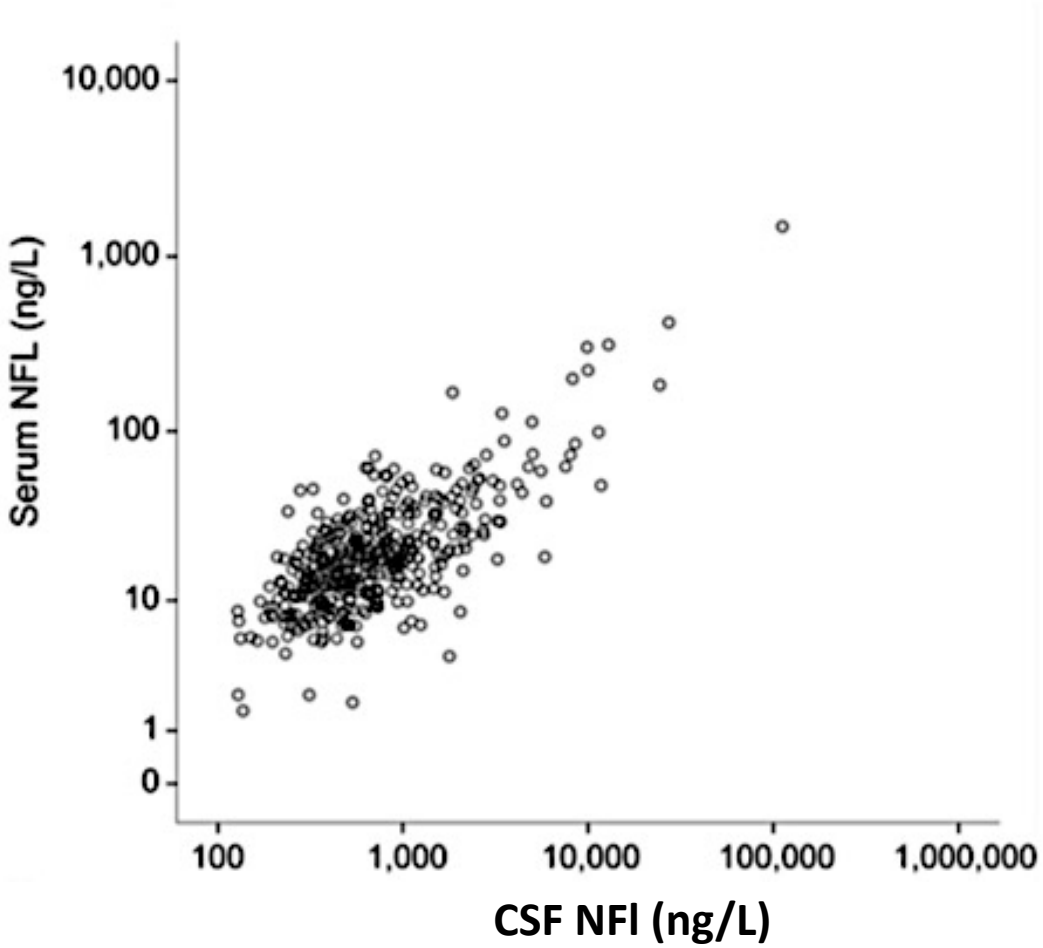
- Conventional ELISA

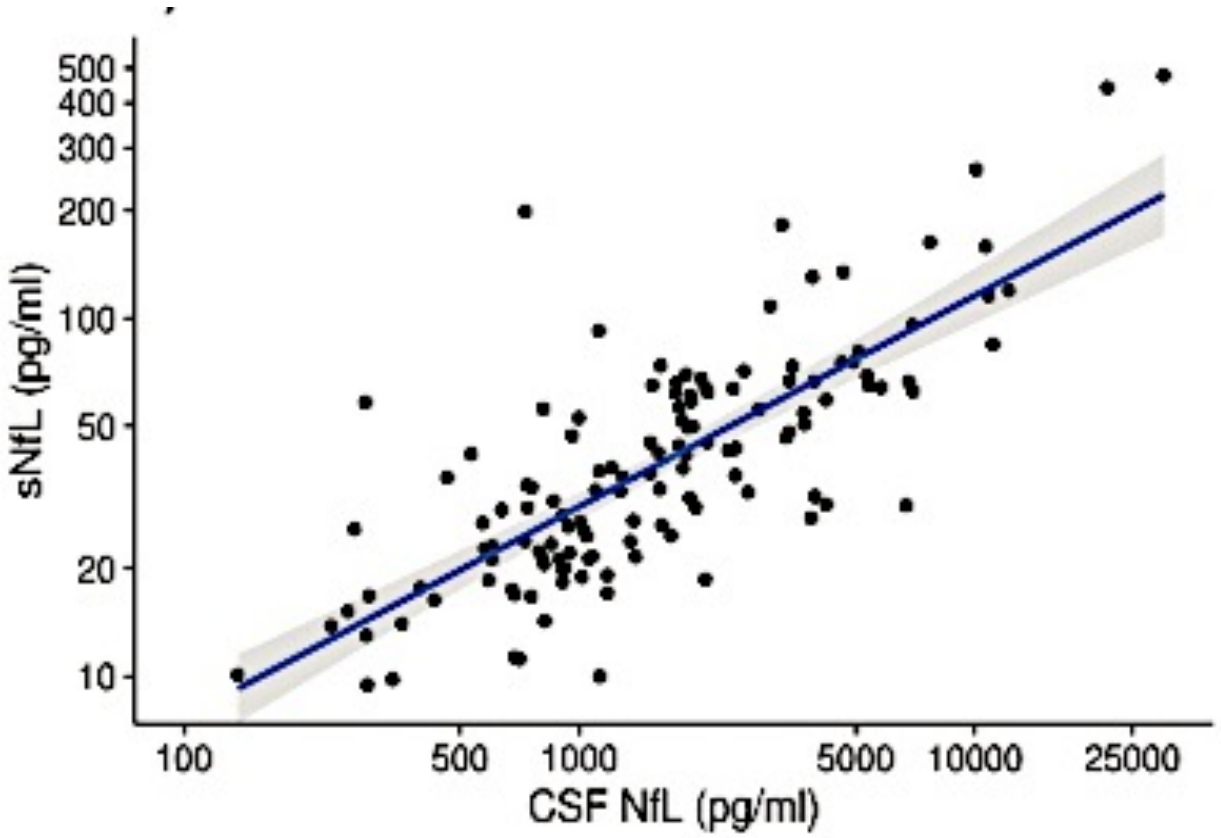
- Electrochemiluminescence-based method (ECL assay)

- Single-molecule array (Simoa)

Analytical sensitivity was 0.62 pg/mL for Simoa, 15.6 pg/mL for the ECL assay, and 78.0 pg/mL for the ELISA.

Correlation Between Serum And CSF Levels Of NfL





Novakova et al. Neurol 2017;89:2230–2237

Disanto et al. ANN NEUROL 2017;81:857–870

sNfL Specificity

- Serum NFL level is a highly predictive marker of long-term poor neurologic outcome at 24 hours after cardiac arrest. *JAMA Neurol. 2018 Oct 29*
- Serum NFL levels were higher in ALS in comparison to other neurologic diseases except for CJD. A cut-off level of 62 pg/mL discriminated between ALS and all other conditions with 85.5% sensitivity (95% CI 78% to 91.2%) and 81.8% specificity (95% CI 74.9% to 87.4%). *J Neurol Neurosurg Psychiatry. 2018 Oct 11. pii: jnnp-2018-318704*.
- Serum NfL holds promise as a biomarker for monitoring primary and secondary neuroaxonal injury after ischemic stroke and for predicting functional outcome. *Neurology. 2018 Sep 14*
- serum NfL correlates with functional impairment and brain atrophy in bvFTD at different disease stages. *Neurology. 2018 Sep 12*

Effect of Ageing On sNfL

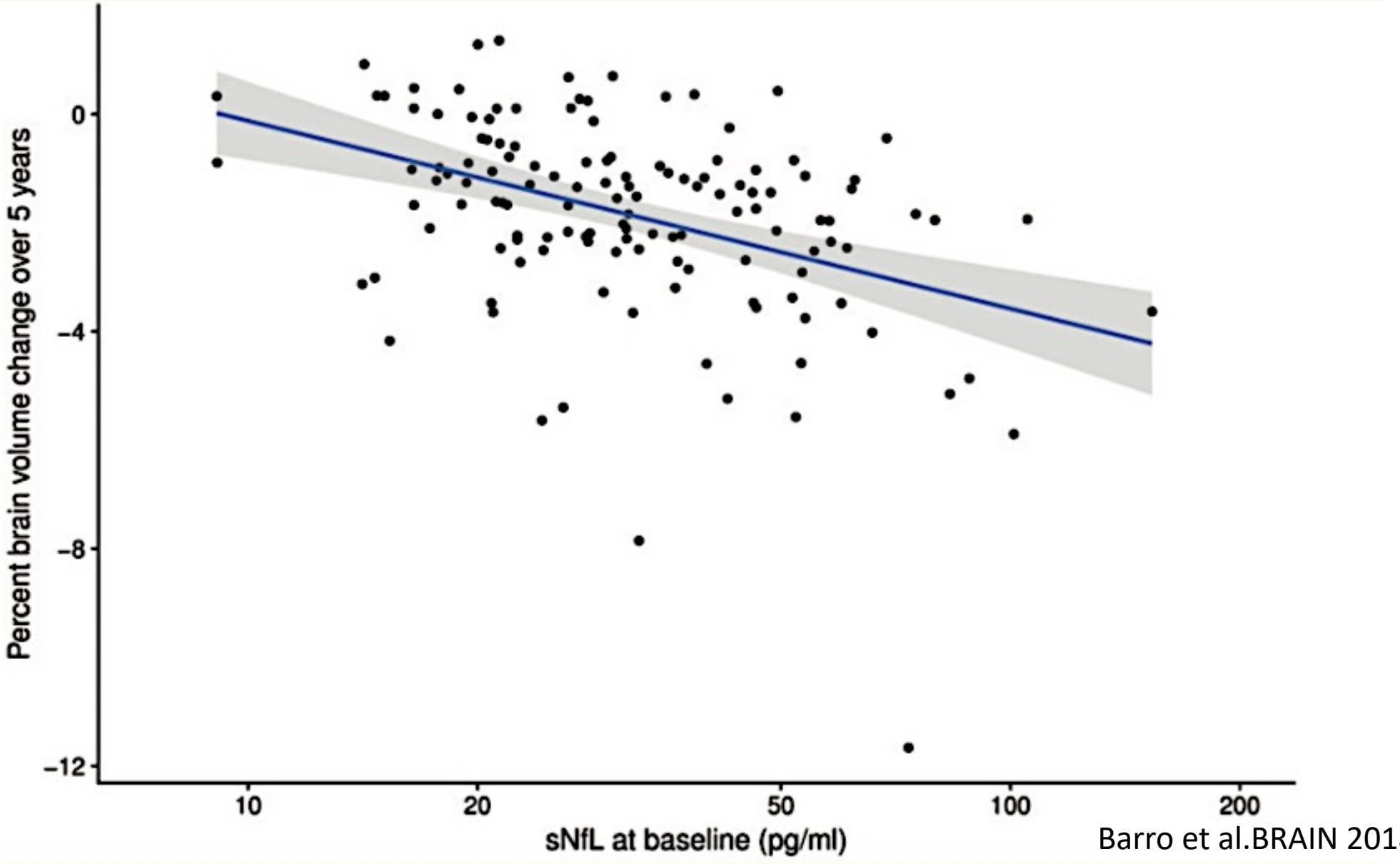
Age, yr	sNfL Percentiles, pg/ml				
	80th	90th	95th	97.5th	99th
30	20.9 (19.3–22.4)	24.3 (22.3–26.3)	27.9 (25.1–30.4)	31.6 (27.6–35.7)	37.2 (30.9–44.4)
35	23.3 (21.9–24.9)	27.1 (25.3–29.2)	31.1 (28.6–34.0)	35.2 (31.7–39.6)	41.5 (35.8–49.4)
40	26.0 (24.7–27.5)	30.3 (28.6–32.3)	34.7 (31.9–37.8)	39.3 (35.4–44.0)	46.3 (40.1–54.9)
45	29.1 (27.7–30.7)	33.9 (32.2–35.9)	38.9 (36.1–41.9)	44.1 (39.8–49.2)	51.9 (44.8–61.5)
50	32.7 (31.1–34.8)	38.1 (35.9–40.3)	43.6 (40.7–47.0)	49.5 (44.7–55.4)	58.3 (50.3–69.4)
55	36.5 (34.2–39.2)	42.5 (39.7–45.4)	48.7 (45.4–52.5)	55.2 (50.4–61.6)	65.0 (56.2–77.3)
60	40.5 (37.7–44.0)	47.2 (43.6–51.0)	54.0 (49.6–58.8)	61.3 (55.4–68.1)	72.1 (62.3–85.1)
65	44.6 (41.0–49.1)	52.0 (47.3–57.1)	59.5 (53.4–65.8)	67.5 (60.0–75.9)	79.5 (68.2–93.4)
70	48.8 (44.2–54.3)	56.9 (51.1–63.4)	65.1 (57.2–73.2)	73.9 (64.3–84.0)	87.0 (73.8–102.7)

sNfL = serum neurofilament light chain.

2.2% increase in sNfL for each additional year ($\beta = 1.022$, 95% CI=1.018– 1.026, $p < 0.001$)
no statistically significant difference between males and females
no association between sNfL and storage time

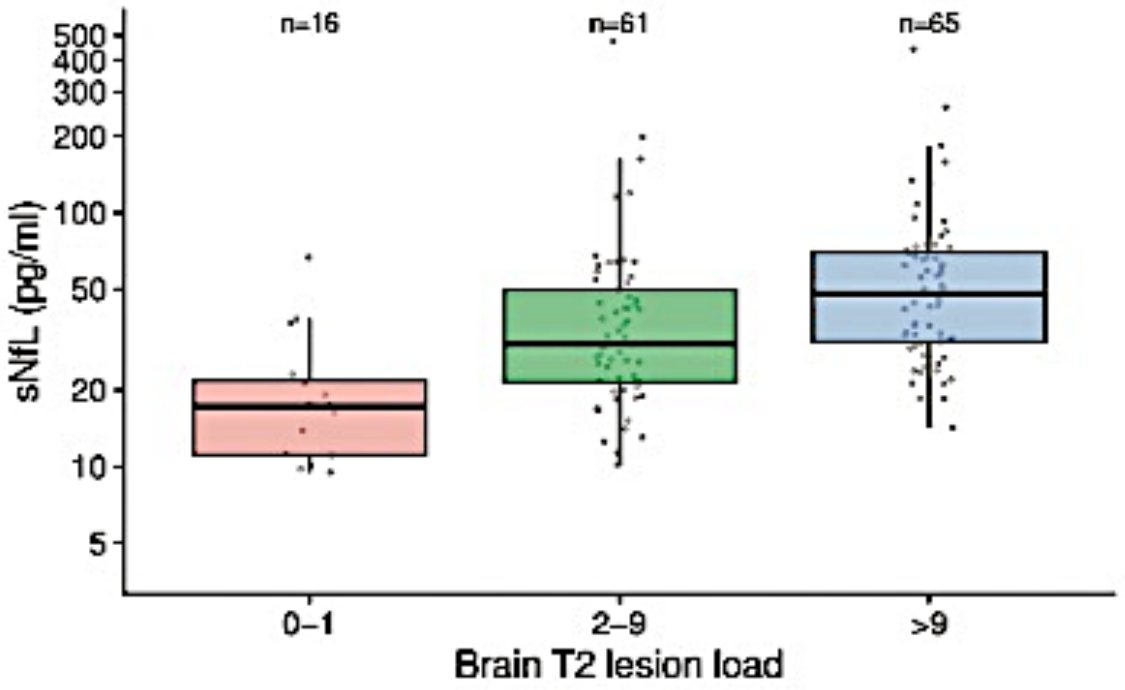
Disanto et al. ANN NEUROL 2017;81:857–870

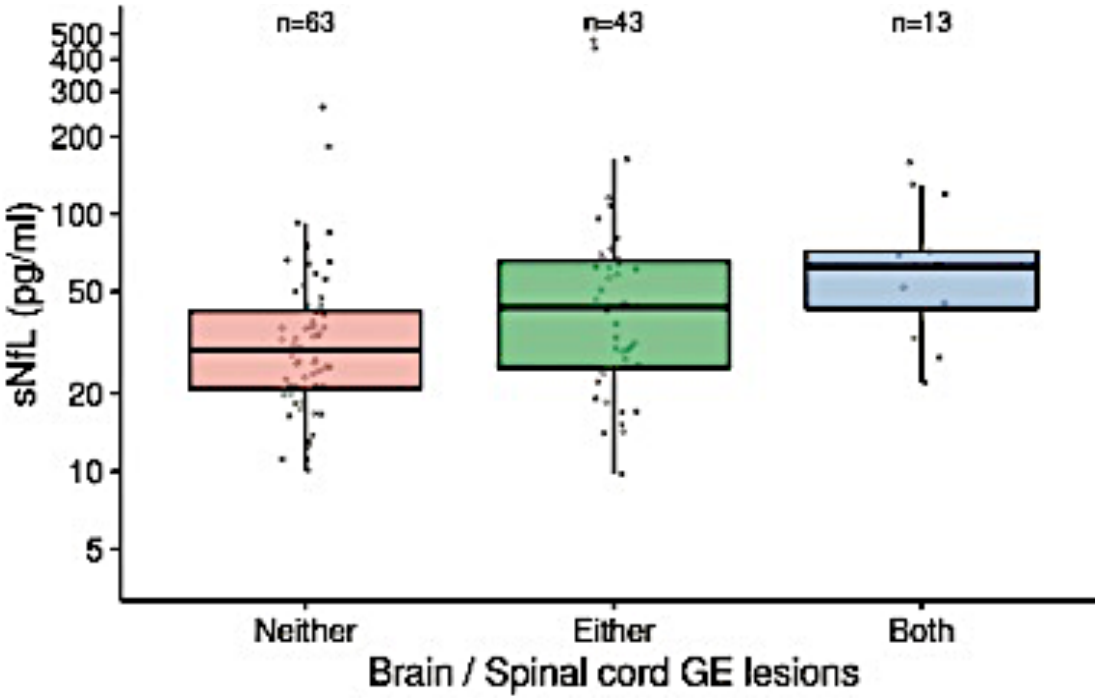
Association of sNfL at baseline with percentage change in brain volume over 5 years



Barro et al.BRAIN 2018: 141; 2382–2391

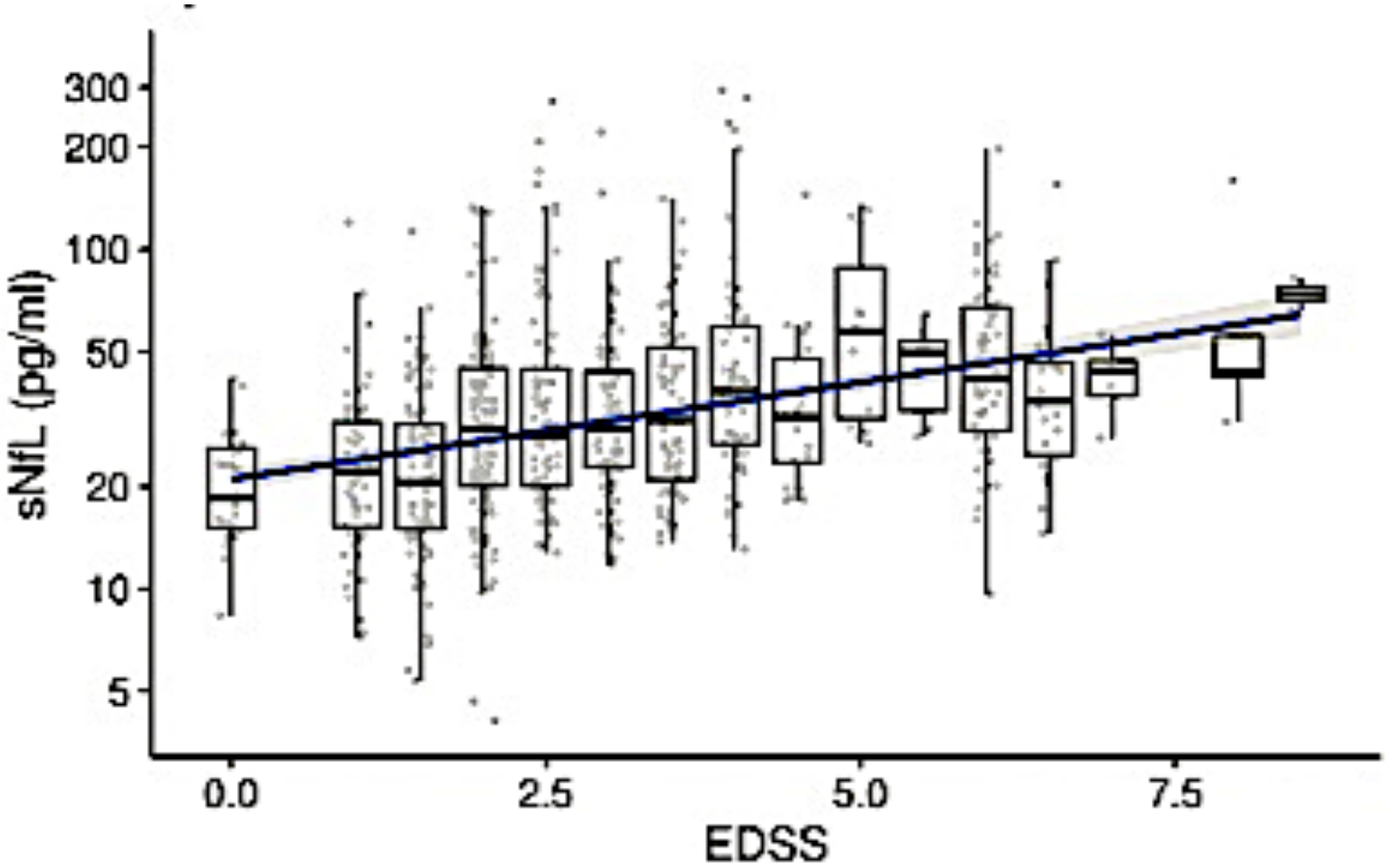
Association Between sNfL levels And MRI





Disanto et al. ANN NEUROL 2017;81:857–870

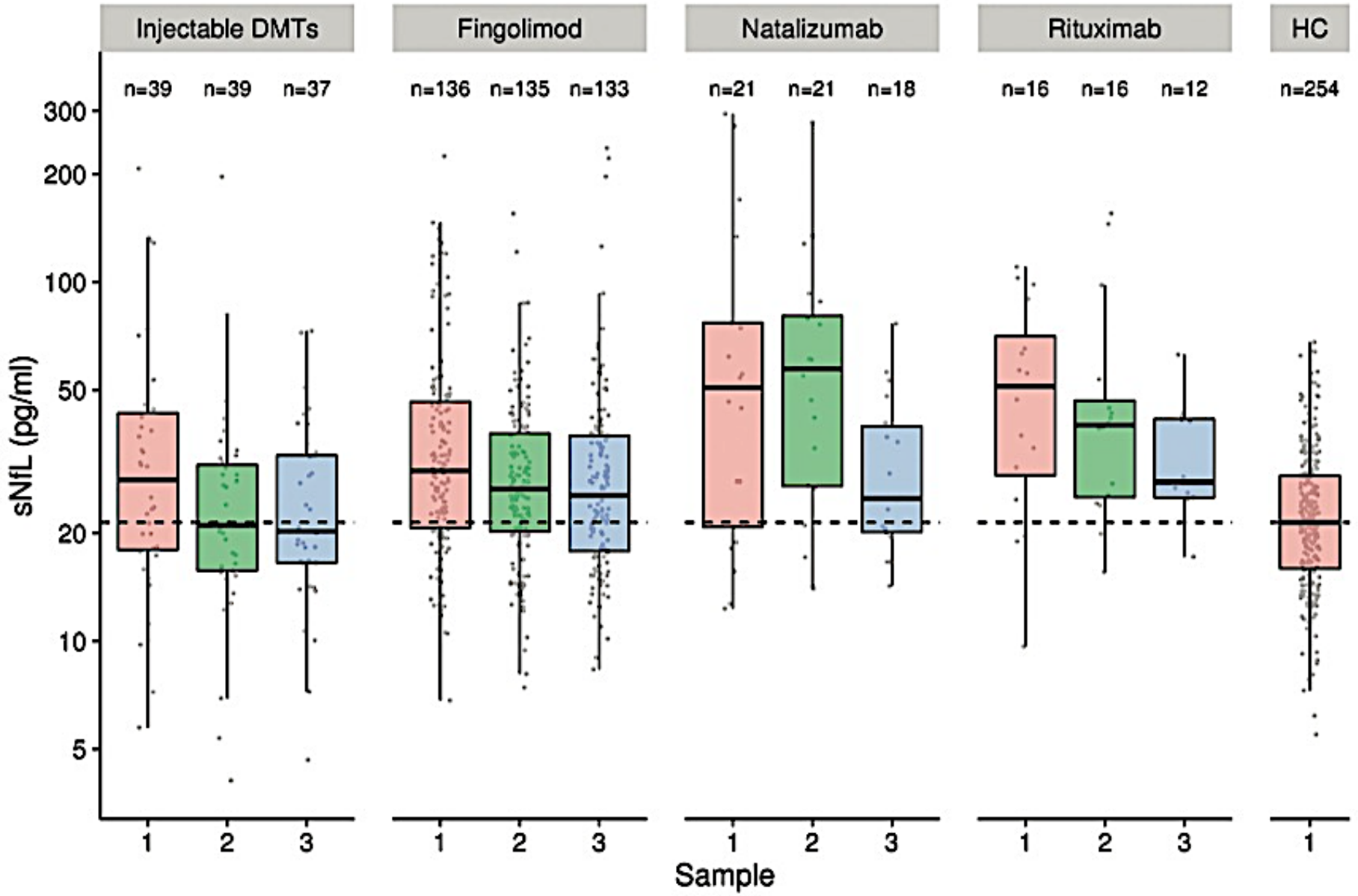
Association Between EDSS And sNfL



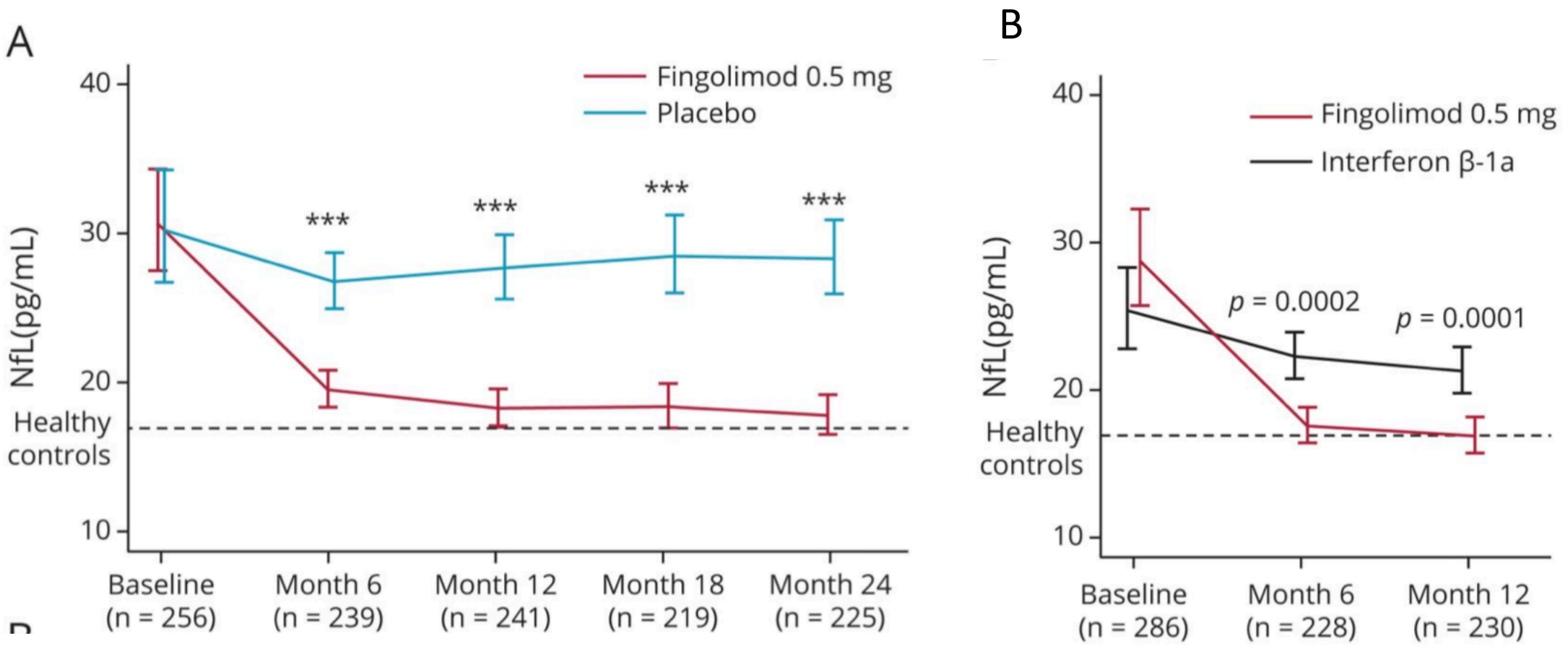
1-point EDSS increase corresponds to an sNfL increase of approximately 14.1%

Disanto et al. ANN NEUROL 2017;81:857–870

Change in sNfL After Treatment



Effect of fingolimod on NfL levels in blood, (A) compared with placebo, FREEDOMS study; (B) compared with interferon-β-1a, TRANSFORMS study



Prognostic potential of baseline NfL for future disease activity and worsening

Irrespective of treatment, patients with high blood NfL concentrations (>60 pg/mL) at baseline compared with those with low baseline NfL concentrations (<30 pg/mL) had 2.6 times more new or enlarging T2 lesions (difference: 164%), 2.5 times more MS relapses (difference: 153%), 2.9 times more brain volume loss (difference: 195%) (all $p < 0.001$), and had a 1.9 times higher risk of 3-month CDW ($p = 0.0605$)

Conclusions

(1) sNfL levels can be reliably and reproducibly measured in serum samples from MS patients

(2) in independent HC and patient cohorts, sNfL levels are positively associated with age but not gender

(3) sNfL levels closely reflect NfL concentration in the CSF of MS patients

(4) sNfL levels are increased in MS patients as compared to HC and positively associated with T2 and GE lesions in both brain and spinal cord

(5) sNfL levels are increased in patients with recent relapses or worsening of disability, are higher with increasing EDSS scores, and decrease with increasing duration of DMT

(6) sNfL levels are associated with an increased risk of future relapses and EDSS worsening.

Questions that need to be answered in MS

Is it possible to monitor individual treatment responses via sNfL?

Can we predict neuronal loss or even transition into long-term progressive disease course in the very early stage of disease and match immunomodulatory therapy regimes?

What do we need before implementing sNfL measurement in clinical practice

More data and research will be needed to establish reference ranges in the general population

Sensitivity and specificity of NfL-based predictions, by using larger cohorts of controls, and taking into account relevant comorbidities and treatment effects.

Assay protocols will need to be standardized and validity of the assay will need to be tested across different populations

Neurology. 2018 Sep 12

References

1. Teunissen CE, Malekzadeh A, Leurs C, Bridel C, Killestein J. Body fluid biomarkers for multiple sclerosis—the long road to clinical application. Nature Reviews Neurology. 2015 Sep 22;:1–12.
2. Comabella M, Montalban X. Body fluid biomarkers in multiple sclerosis. Lancet Neurol. 2014 Jan;13(1):113–26.
3. Comabella M, Fernández M, Martin R, Rivera-Vallvé S, Borrás E, Chiva C, et al. Cerebrospinal fluid chitinase 3-like 1 levels are associated with conversion to multiple sclerosis. Brain. 2010.
4. Khalil M, Teunissen CE, Otto M, Piehl F, Sormani MP, Gattringer T, et al. Neurofilaments as biomarkers in neurological disorders. Nature Reviews Neurology. Springer US; 2018 Sep 26;:1–13.
5. Novakova L, Zetterberg H, Sundström P, Axelsson M, Khademi M, Gunnarsson M, et al. Monitoring disease activity in multiple sclerosis using serum neurofilament light protein. Neurology. Lippincott Williams & Wilkins; 2017 Oct 27;89(22):2230–7.
6. Disanto G, Barro C, Benkert P, Naegelin Y, Schädelin S, Giardiello A, et al. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. Ann Neurol. 2017 Jun;81(6):857–70.
7. Barro C, Benkert P, Disanto G, Tsagkas C, Amann M, Naegelin Y, et al. Serum neurofilament as a predictor of disease worsening and brain and spinal cord atrophy in multiple sclerosis. Brain. Oxford University Press; 2018 Aug 1;141(8):2382–91.
8. Cantó E, Barro C, Zhao C, Caillier SJ, Michalak Z, Bove R, et al. Association Between Serum Neurofilament Light Chain Levels and Long-term Disease Course Among Patients With Multiple Sclerosis Followed up for 12 Years. JAMA Neurol. 2019 Aug 12;:1–8.
9. Kuhle J, Nourbakhsh B, Grant D, Morant S, Barro C, Yaldizli Ö, et al. Serum neurofilament is associated with progression of brain atrophy and disability in early MS. Neurology. American Academy of Neurology; 2017 Feb 28;88(9):826–31.