CONVEGNO REGIONALE SIN /SNO

Liguria - Piemonte e Valle d'Aosta

Ivrea, 6-7 dicembre 2019

Università infermieristica di Ivrea

Antonio Bertolotto: disclosures

Type of affiliation or financial support	Name of organization
Contribution in research	2017: Almirall, Bayer, Biogen, Genzyme, Merck, Novartis, TEVA, FISM 2018: Almiral, Biogen, Novartis, TEVA, FISM, 2019: Almiral, Biogen, Genzyme, Novartis, Merck, Sanofi, FISM
Consultancy activity	2017: Roche, Sanofi 2018: Biogen, Sanofi 2019: Biogen, Novartis, Roche, Sanofi Genzyme
Activity of lecturer	2017: Biogen, Novartis, Sanofi 2018: Biogen, Mylan, Novartis, Santhera, Sanofi, TEVA 2019: Biogen, Mylan, Novartis, Roche, Sanofi Genzyme, Santhera
Possession of shares	/

THE SIMOA TECHNOLOGY

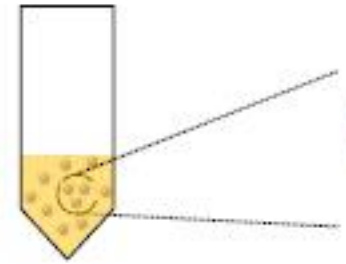
Sensitivity

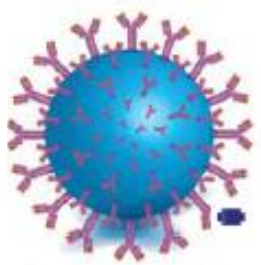
ELISA assay **1970-2000** (ng/mL)

ECL assays **2000-2014** (pg/mL)

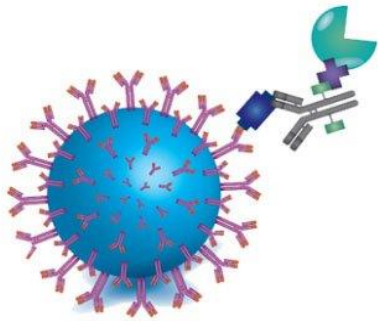
SIMOA **2014+** (fg/mL)

SIMOA BEAD ASSAYS

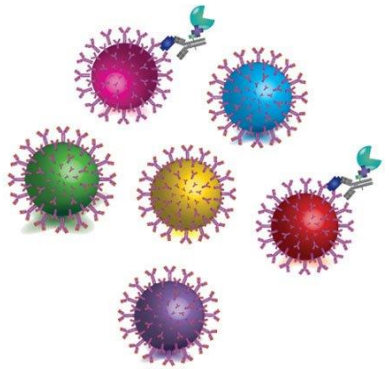




500000 paramagnetic beads coupled with capture antibodies specific for each target are added to the sample

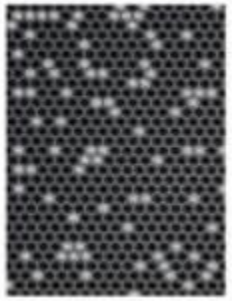


Formation of an immunocomplex consisting of the bead, bound protein, and detection antibody.

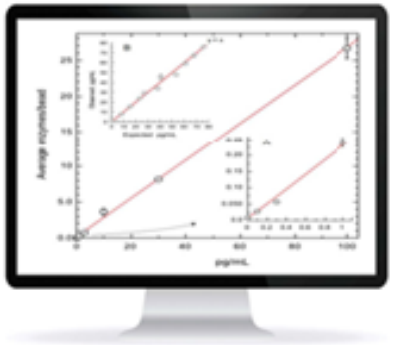


Each bead will contain bound proteins, or not

The sample is loaded onto arrays, consisting of 250,000 microwells, each large enough to hold one bead

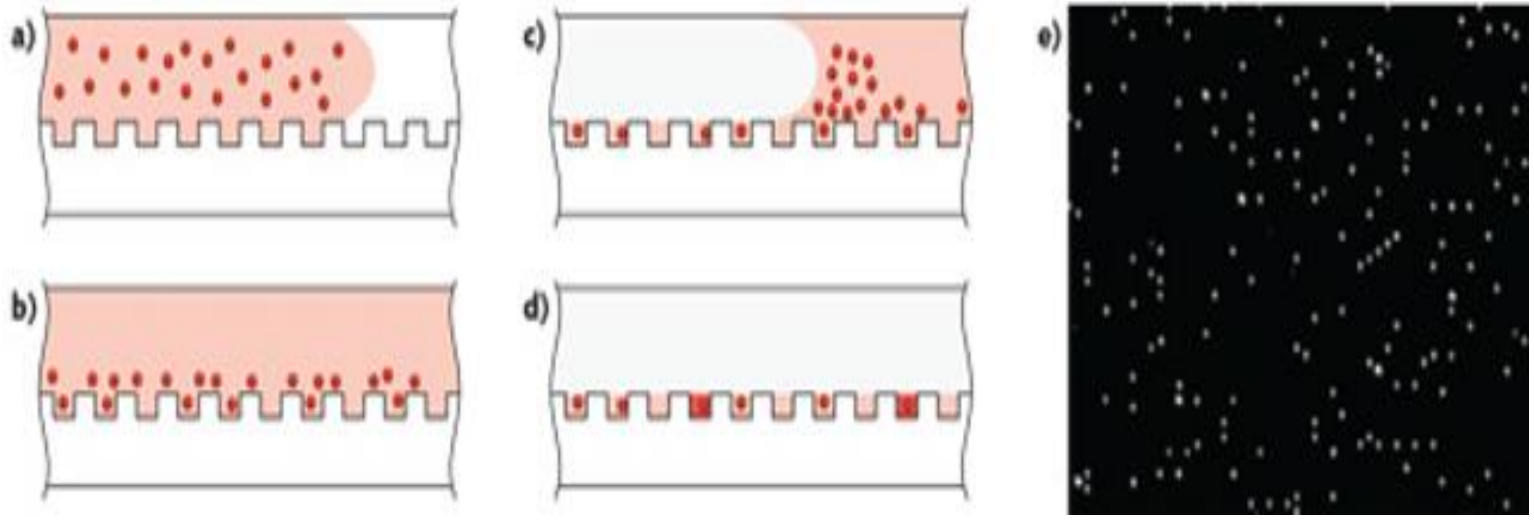


Fluorescence imaging



Data analysis

Single Molecule Array



Array

Simoa utilizza 24 arrays (contenitori) posizionati in cerchio in ciascuno dei quali ci sono 216 000 piccoli ‘’pozzetti’’ che possono contenere sono 1 elemento da analizzare (single molecule)

24 x 216 000

Applications of SIMOA technology

- Analysis of biomarkers previously difficult or impossible to be measured
- Different biological substrates: serum, plasma, cerebrospinal fluid, cellular extracts.
- Fields of application:

- Hundreds of international pubblications are available, showing the great interest towards this technology and its applications.

> 50 pubblicazioni nel 2019 su PubMed con la ricerca «SIMOA»

Available SIMOA assays


ONCOLOGY


NEUROLOGY


CARDIOLOGY


INFLAMMATORY


INFECTIOUS DISEASE

Advantage Assays - Human

Analyte	LoD (pg/mL)	LoQ (pg/mL)	Dynamic Range (pg/mL)	Median Endogenous (pg/mL)	Sample Volume*	Sample Type**	Catalog Number
Aβ40	0.522	1.23	0-800	65.97	32.5 µL	C, E	101672
Aβ42	0.044	0.137	0-400	4.7	32.5 µL	C, E	101664
C-Peptide	0.013	0.021	0-400	1559	25 µL	E, S	100199
Eotaxin / CXCL11	0.1	0.18	0-960	75.6	13 µL	E, S	101212
G-CSF	0.095	0.095	0-400	7.12	25 µL	E, S	101235
GM-CSF	0.0019	0.0103	0-120	0.0865	33 µL	E, S	102329
IFNα	0.0025	0.0047	0-150	0.0036	73 µL	E, S	100860
IFNγ	0.0104	0.0764	0-400	0.333	32.5 µL	E, S	100200
IL-1β	0.016	0.083	0-240	0.058	100 µL	E, S	101605
IL-2	0.011	0.041	0-120	0.086	49.5 µL	E, S	101635
IL-4	0.0046	0.039	0-200	0.024	65 µL	E, S	100196
IL-5	0.004	0.0165	0-12	0.22	76 µL	E, S	102860
IL-6	0.0055	0.01	0-120	1.73	32.5 µL	E, S	101622
IL-7	0.009	0.103	0-600	28.0	25 µL	E, S	103277
IL-8	0.056	0.0921	0-1,200	5.31	25 µL	E, S	100198
IL-10	0.0038	0.021	0-120	0.94	32.5 µL	E, S	101643
IL-12p70	0.0048	0.017	0-40	1.95	25 µL	E, S	100988
IL-13	0.002	0.005	0-30	0.039	65 µL	E, S	102732
IL-15	0.003	0.0062	0-40	3.23	25 µL	E, S	100794
IL-17A	0.0042	0.021	0-120	0.124	32.5 µL	E, S	101599
IP-10	0.052	0.177	0-800	105	25 µL	E, S	101132
MCP-1	N/A	0.153	0-800	85.3	25 µL	E, S	101154
NF-light®	0.038	0.174	0-2,000	5.33	46 µL	C, E, S	102258
HIV p24	0.0027	0.01	N/A	N/A	154 µL	E, S	102215
PSA	0.015	0.024	0-400	1.81	32.5 µL	E, S	101478
Tau	0.019	0.061	0-360	1.65	45.5 µL	C, E, S	101552
P-Tau 231	0.621	1.83	0-1,200	20.8	38 µL	C	102292
TDP-43	2.48	8.23	0-8,000	130	25 µL	C, E, S	103293
TNF-α	0.016	0.034	0-400	1.94	32.5 µL	E, S	101580
TRAIL	0.0083	0.0177	0-400	23.1	25 µL	E, S	100906
Troponin-I	0.013	0.079	0-1,200	0.646	49.5 µL	E, S	101588

Discovery Assays – Human

Analyte	LoD (pg/mL)	LoQ (pg/mL)	Dynamic Range (pg/mL)	Median Endogenous (pg/mL)	Sample Volume†	Sample Type††	Catalog Number
α-Synuclein	0.955	4.12	0–10,000	4,145	19 µL	C, E, S	102233
BDNF	0.011	0.034	0–64,000	11,306	33 µL	C, E, S	102039
CA 19-9	0.023 U/mL	0.41 U/mL	0–240 U/mL	0.83 U/mL	106 µL	E, S	102543
CA-125	0.003 U/mL	0.010 U/mL	0–200 U/mL	1.62 U/mL	13.65 µL	E, S	102136
Cathepsin S	0.7	1.95	0–200	6,566	10 µL	E, S	102064
CEA	0.486	2.33	0–85 ng/mL	1511.5	10 µL	E, S	102556
c-MET	0.036	0.244	0–800	58,012	10 µL	E, L, S	102073
CRP	0.048	0.686	0–48	19	10 µL	E, S	102583
CXCL13	0.048	0.07	0–800	22.63	33 µL	E, S	102635
GFAP*	0.211	0.686	0–4,000	88	46 µL	C, E, S	102336
HE4 / WFDC2	0.135	0.977	0–4,000	104	55 µL	E, S	103059
IL-1α	0.004	0.01	0–60	0.0293	65 µL	E, S	101968
IL-3	0.226	0.686	0–2000	0.279	46 µL	E, S	102462
IL-12p40/IL-23	0.02	0.086	0–1,000	51.3	33 µL	E, S	101871
IL-17C	0.065	0.206	0–1,200	1.66	33 µL	E, S	102570
IL-18	0.004	0.012	0–2250	200.1	10 µL	C, E, S	102700
IL-22 (Total)	0.0054	0.0103	0–120	7.16	55 µL	E, S	103071
IL-23	0.132	0.686	0–2,000	0.31	90 µL	E, S	102184
IL-28A	0.022	0.069	0–200	0.303	65 µL	E, S	101419
IL-33	0.32	0.686	0–2,000	5.45	65 µL	E, S	103093
IL-36β	0.01	0.206	0–600	0.426	33 µL	E, S	101808
Leptin	2.46	4.94	0–60,000	6,083	2 µL	E, S	101855
LIF	0.015	0.086	0–520	0.412	33 µL	E, S	102394
MCP-3	0.124	0.309	0–450	0.445	75 µL	E, S	102382
MIP-1β	0.034	0.137	0–800	66.7	46 µL	E, S	102599
MMP-9	0.581	4.88	0–5,000 ng/ml	555	1 µL	C, E, S	102491
pNF-heavy	0.663	2.88	0–8,400	30.82	13 µL	C, E, S	102669
NSE	1.296	9.88	0–120	7,845	2 µL	C, E, S	102475
NT-proBNP	0.043	0.206	0–500	71	22 µL	E, S	102713
PD-1	0.247	0.879	0–7200	73	58 µL	E, S	102929
PD-L1	0.055	0.617	0–4,300	33.79	10 µL	E, S	102648
PlGF	0.064	0.3	0–960	3.82	38 µL	E, S	102318
TGFα	0.031	0.207	0–900	3.34	65 µL	E, S	101863
TGFβ	0.137	0.514	0–24,000	34,836	8.5 µL	E, S	101984
TNFβ	0.052	0.15	0–2,400	7.168	55 µL	E, S	102091
UCH-L1*	1.05	3.43	0–20,000	9.51	46 µL	C, E, S	102343
VEGF	0.041	0.137	0–800	3.49	33 µL	E, S	102794

Oncology

PD1 and PD-L1 dosage to distinguish PD-1 inhibitor nonresponders as early as after one dose after therapy and applications in characterizing PD-1 inhibitor resistance

Biomarkers for Immunotherapy of Cancer pp 399-412 | Cite as

Single-Molecule Arrays for Ultrasensitive Detection of Blood-Based Biomarkers for Immunotherapy

Authors | Authors and affiliations

Limor Cohen, Alissa Keegan, David R. Walt

C-MET and AREG dosage in advanced rectal cancer

Locally advanced rectal cancer transcriptomic-based secretome analysis reveals novel biomarkers useful to identify patients according to neoadjuvant chemoradiotherapy response

Luisa Matos do Canto, Sarah Santiloni Cury, Mateus Camargo Barros-Filho, Bruna Elisa Catin Kupper, Maria Dirlei Ferreira de Souza Begnami, Cristovam Scapulatempo-Neto, Robson Francisco Carvalho, Fabio Albuquerque Marchi, Dorte Aalund Olsen, Jonna Skov Madsen, Birgitte Mayland Havelund, Samuel Aguiar Jr. & Silvia Regina Rogatto

Cardiology

Circulating cardiac Troponin 1. Correlation with age and sex, implications for risk stratification purposes

Abstract 19167: Ultra-high Sensitive Cardiac Troponin I Baseline Levels are Affected by Age and Sex

Mitra Mastali, Qin Fu, Kimia Sobhani, Noel Bairey Merz, and Jennifer Van Eyk

Originally published 9 Jun 2018 | Circulation. 2017;136:A19167

RBM3, new candidate as a biomarker for therapeutic hypothermia and a possible new therapeutic target for organ protection

A Prospective Clinical Trial Measuring the Effects of Cardiopulmonary Bypass Under Mild Hypothermia on the Inflammatory Response and Regulation of Cold-Shock Protein RNA-Binding Motif 3

Lisa-Maria Rosenthal ✉, Giang Tong, Sylvia Wowro, Christoph Walker, Constanze Pfitzer, Wolfgang Böttcher, Oliver Miera, Felix Berger, and Katharina Rose Luise Schmitt

Immunology and inflammation

IFN-alfa. Evaluation of treatment efficacy in LES patients

Control of TLR7-mediated type I IFN signaling in pDCs through CXCR4 engagement—A new target for lupus treatment

Nikaïa Smith[1,2,3,4]*, Mathieu P. Rodero[1,2,3], Nassima Bekaddour[1,2,3], Vincent Bondet[5,6],

IFN-alfa. High levels correlates with a higher risk of relapse

Ultrasensitive serum interferon-α quantification during SLE remission identifies patients at risk for relapse

Alexis Mathian[1], Suzanne Mouries-Martin[2], Karim Dorgham[3], Hervé Devilliers[4], Hans Yssel[3], Laura Garrido Castillo[3], Fleur Coh
Aubart[1], Julien Haroche[1], Miguel Hié[1], Marc Pineton de Chambrun[1], Makoto Miyara[3], Micheline Pha[1], Flore Rozenberg[5], Guy
Gorochov[3], Zahir Amoura[1]

Infectious Disease assays

IL-6, IL-8, IL-18, and VEGF. A blood-based host response panel can help to differentiate active tubercolosis from other causes of persistent cough in patients with and without HIV infection.

A rapid triage test for active pulmonary tuberculosis in adult patients with persistent cough

Rushdy Ahmad[1,*], Liangxia Xie[2,3,4], Margaret Pyle[5], Marta F. Suarez[6], Tobias Broger[7], Dan Steinberg[8], Shaali M. Ame[9], Maril...

+ See all authors and affiliations

Botulinum neurotoxin serotype A1. Quantitative analysis of **BoNT/A1**, also at low amount in seum samples

Rapid and ultrasensitive detection of botulinum neurotoxin serotype A1 in human serum and urine using single-molecule array method

Authors | Authors and affiliations

Trinh L. Dinh, Kevin C. Ngan, Charles B. Shoemaker, David R. Walt

Neurology

Neurodegeneration, neuroinflammation, traumatic brain injuries (TBI) and multiple sclerosis (MS) represent the strategic focus, in which SIMOA technology is finding the major advancements in health research and precision health medicine.

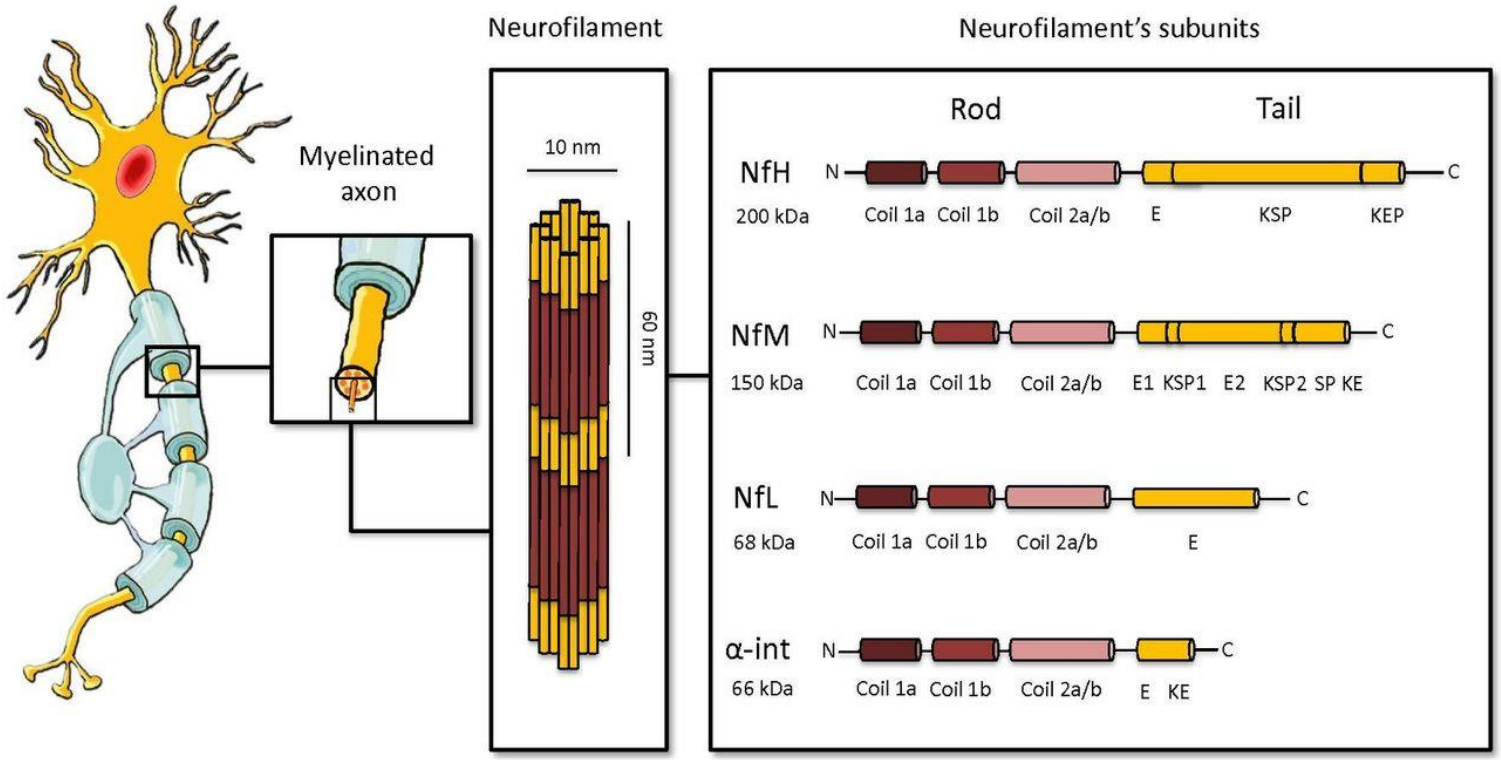
Alpha-synuclein	GFAP*, UCHL-1*)
Aβ40	NF-light®
Aβ42	NF-light® Advantage Kit (SR-X)
BDNF	NSE
GFAP	P-Tau 181
MMP-9	P-Tau 231
Neuro 4-Plex B	pNF-Heavy
Neurology 2-Plex A (Tau, Aβ42)	Tau
Neurology 3-Plex A (Tau, Aβ42, Aβ40)	Tau (mouse)
	TDP-43
Neurology 4-Plex A (NF-light®, Tau,	UCH-L1

Multiplex Advantage Assays – Human

Assay	Analytes	LoD (pg/mL)	LoQ (pg/mL)	Dynamic Range (pg/mL)	Median Endogenous (pg/mL)	Sample Volume[†]	Sample Type[††]	Catalog Number
Cytokine 3-Plex A	TNFα	0.011	0.051	0–112	1.44	25 µL	E, S	101160
	IL-6	0.006	0.011	0–60	1.71			
	IL-10	0.0022	0.0073	0–24	0.4			
Cytokine 3-Plex B	TNFα	0.021	0.026	0–112	2.48	25 µL	E, S	101319
	IL-6	0.011	0.023	0–60	1.33			
	IL-17A	0.0047	0.0068	0–40	0.057			
Neurology 2-Plex A	Aβ42	0.0249	0.171	0–800	8.1	18.4	C, E	101876
	Tau	0.02	0.067	0–400	2.75			
Neurology 3-Plex A	Aβ40	0.196	0.675	0–800	209	46 µL	C, E	101995
	Aβ42	0.045	0.142	0–400	11.1			
	Tau	0.019	0.063	0–400	1.43			
Neurology 4-Plex A	GFAP	0.221	0.467	0–4000	89.7	46 µL 4.6 µL (CSF)	C, E, S	102153
	NF-light	0.104	0.241	0–2000	10.6			
	Tau	0.024	0.053	0–400	2.21			
	UCH-L1	1.74	5.45	0–40,000	12.21			

NEUROFILAMENTS

- Structural scaffolding proteins exclusively expressed in neurons
- Highly specific for neuronal cell damage
- Following axonal damage neurofilament proteins are realeased into CSF and peripheral blood (at low concentration)
- High levels of neurofilaments are general indicators of axonal damage



Gaetani L. et al. J Neurol Neurosurg Psychiatry 2019

SHIFT FROM CSF TO SERUM

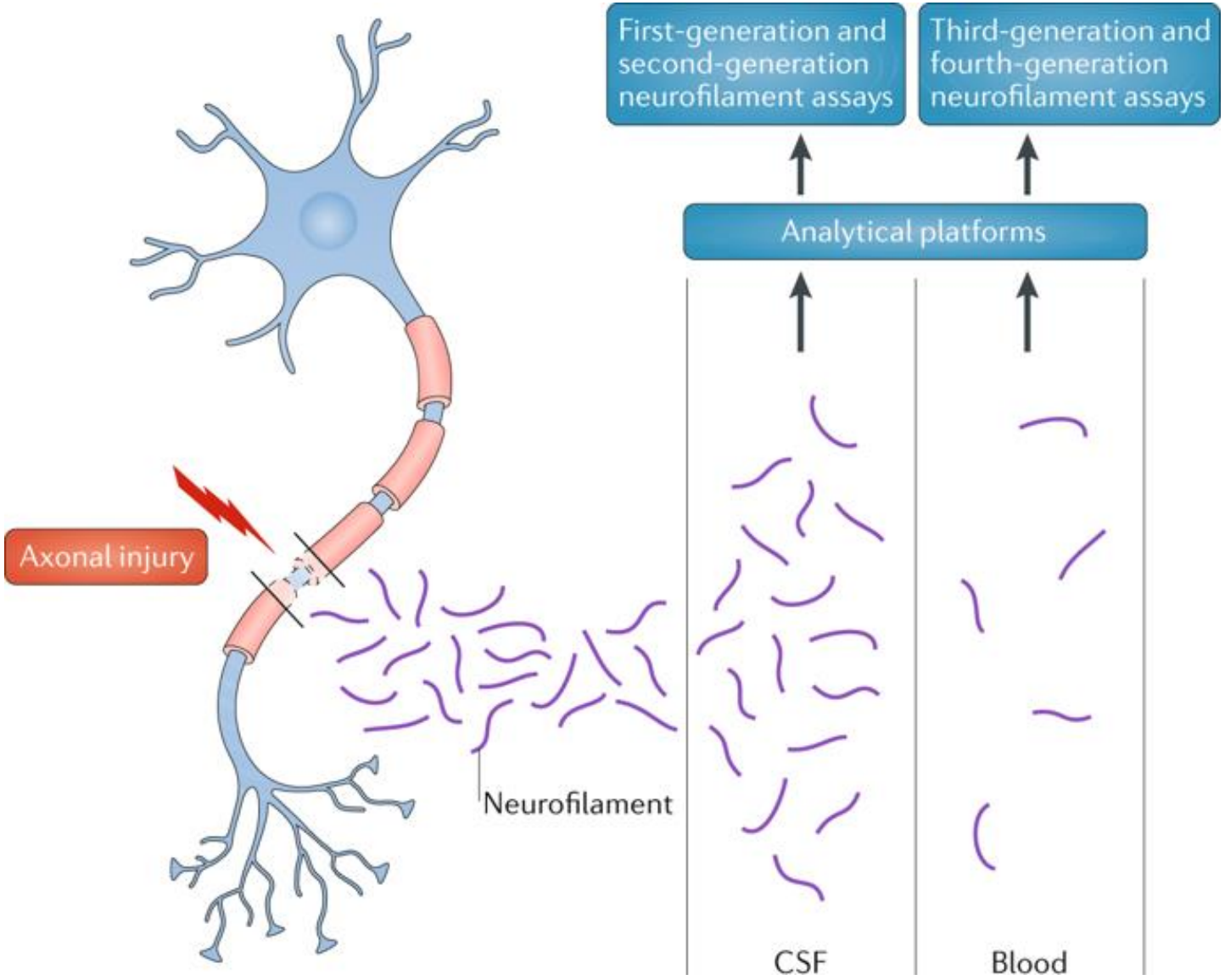
- need for lumbar puncture constitutes a major barrier for more widespread use

- moving from CSF- to blood-based biomarkers would be a major step for **longitudinal studies**.

- Need of **ultrasensitive assays** to detect proteins that are released into the bloodstream at very low concentration

ASSAYS TO DETECT NFL

1. Immunoblot
2. ELISA
3. Electrochemiluminescence (ECL)
4. Single-molecule array (SIMOA)

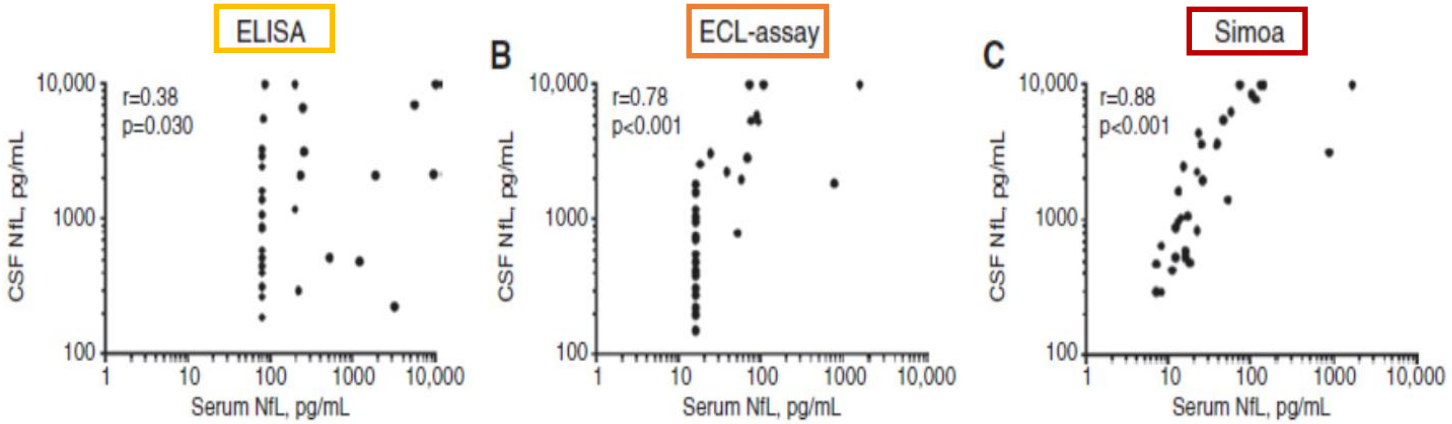


Modified from Khalil et al, 2018

NFL IN SERUM/PLASMA

Jens Kuhle*, Christian Barro, Ulf Andreasson, Tobias Derfuss, Raija Lindberg, Åsa Sandelius, Victor Liman, Niklas Norgren, Kaj Blennow[a] and Henrik Zetterberg[a]

Comparison of three analytical platforms for quantification of the neurofilament light chain in blood samples: ELISA, electrochemiluminescence immunoassay and Simoa



Modified from Kuhle J et al. Clin Chem Lab, 2016

SENSITIVITY

SIMOA sensitivity is 126-fold higher than traditional ELISA and 25-fold more sensitive than ECL assay

SIMOA: 0.62 pg/ml

ECL: 15.6 pg/ml

ELISA: 78.0 pg/ml

NEUROFILAMENTS IN NEUROLOGICAL DISORDERS

Box 1 | Relevance of neurofilaments to neurological disorders

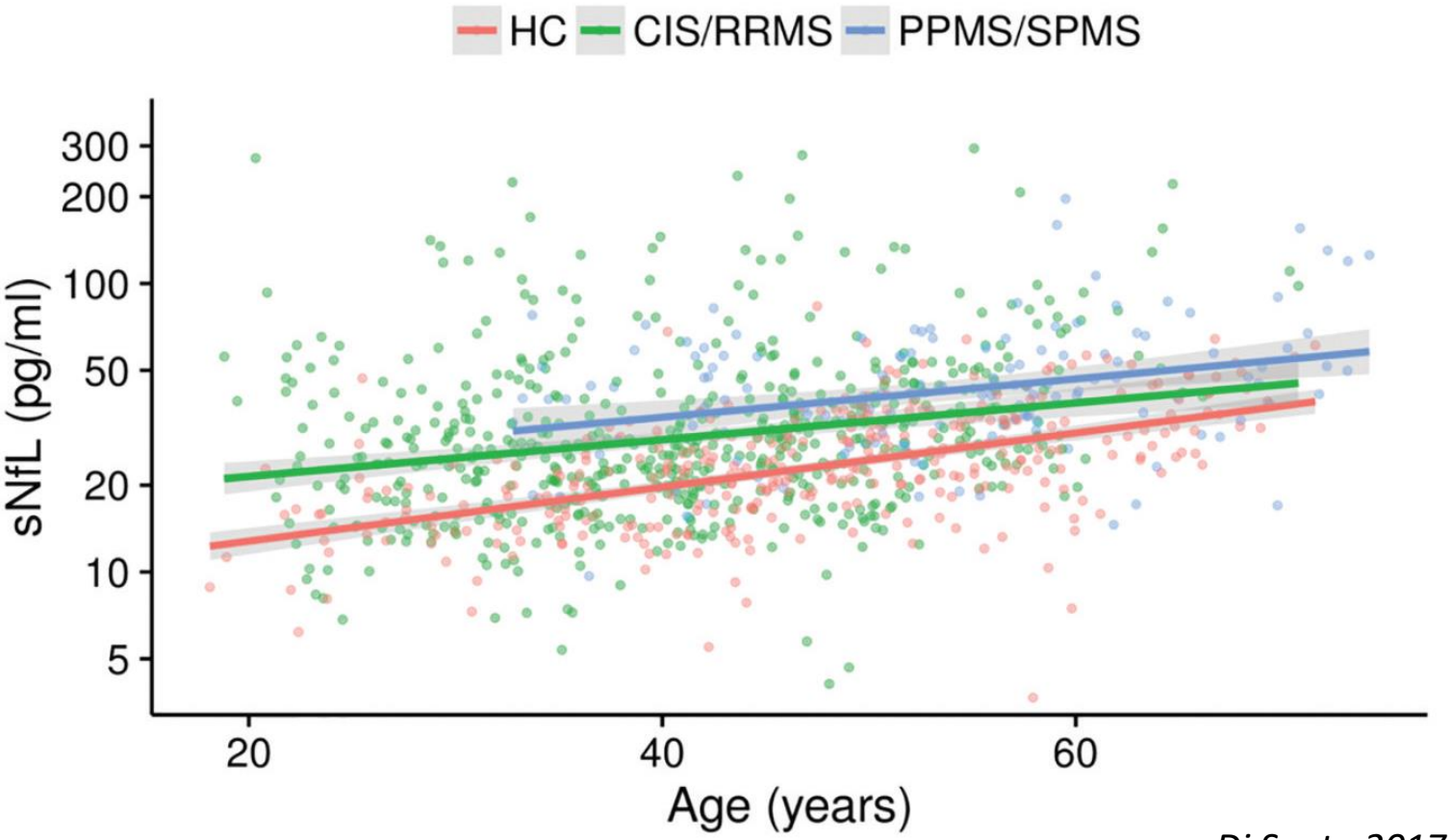
Neurofilaments have been studied in several neurological disorders, and, in many, good evidence supports their diagnostic and prognostic value and/or their use for monitoring treatment responses. The disorders reviewed here are as follows:

- Multiple sclerosis
- Dementia
- Stroke
- Traumatic brain injury
- Amyotrophic lateral sclerosis
- Parkinson disease
- Huntington disease
- Bipolar disorder (limited evidence for clinical utility)

In addition, neurofilaments could be of relevance in many other neurological disorders, but their association with these disorders has not systematically been studied. Such disorders include the following:

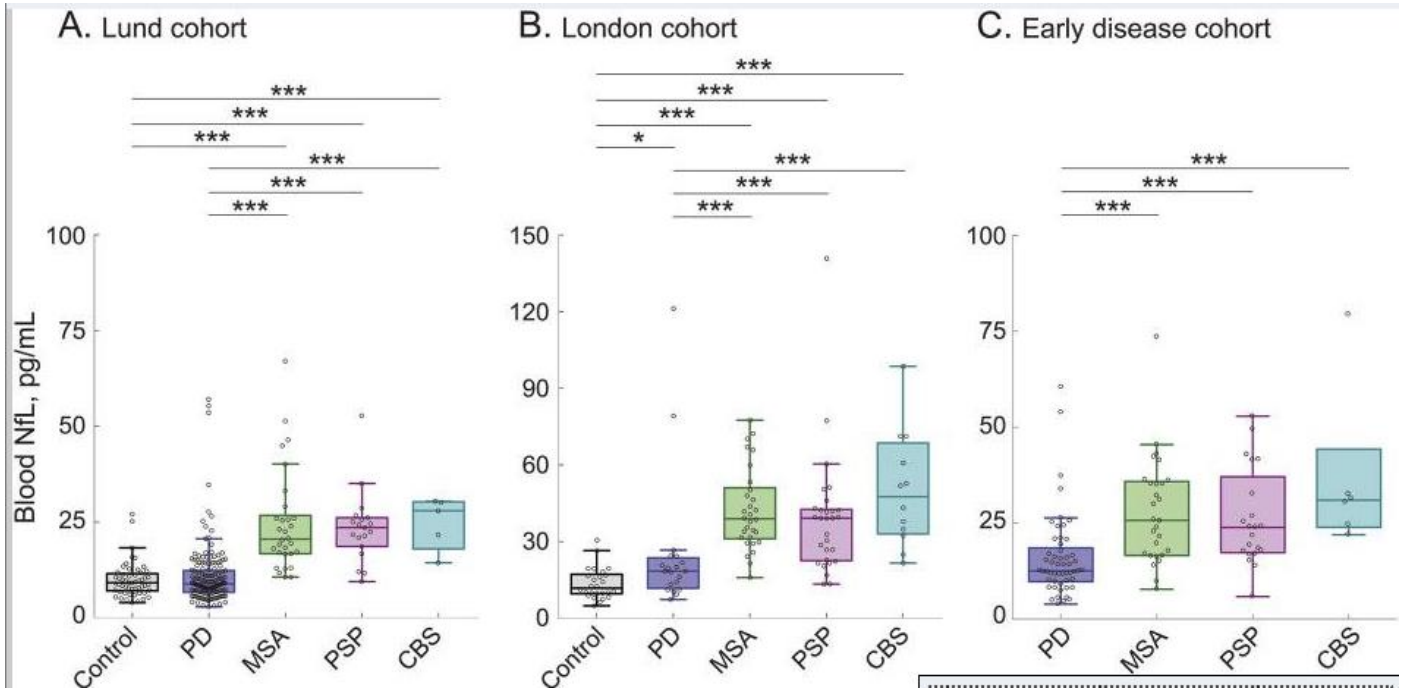
- Epilepsy
- Encephalitis
- Meningitis
- Hypoxic brain injury
- Optic neuropathies
- Intracranial pressure
- Neurotoxicity
- Peripheral neuropathies including Guillain–Barré syndrome, chronic inflammatory demyelinating neuropathy and Charcot–Marie–Tooth disease

NFL LEVELS AND AGE



Di Santo 2017

NFL in parkinsonian disorders



- **Findings:**
  - Blood and CSF NfL correlate
  - NfL is significantly increased in APD vs. controls and PD, including early stage disease
  - NfL correlated with disease severity (NOT duration)

- **Conclusion:** NfL has potential value in differentiating PD from APD

Hansson et al, Neurology 2017

ARTICLE CLASS OF EVIDENCE

Blood NfL

A biomarker for disease severity and progression in Parkinson disease

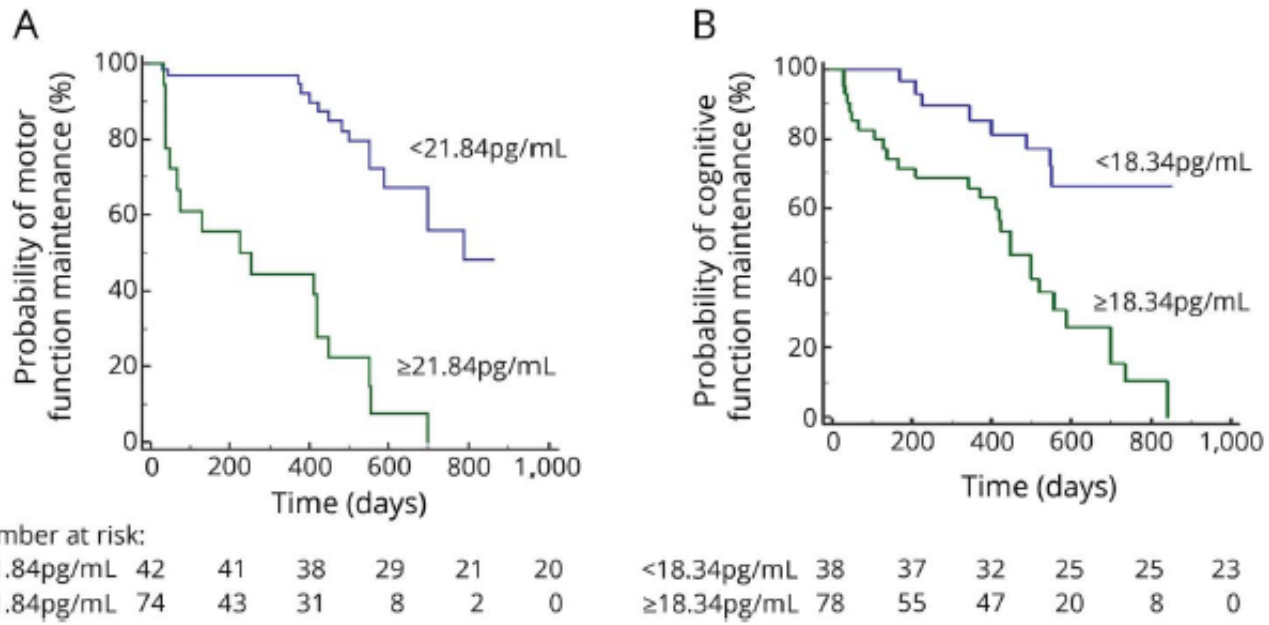
Chin-Hsien Lin, MD, PhD, Cheng-Hsuan Li, MD, Kai-Chien Yang, MD, PhD, Fang-Ju Lin, PhD, Chau-Chung Wu, MD, PhD, Jen-Jie Chieh, PhD, and Ming-Jang Chiu, MD, PhD

Correspondence
Dr. Chiu
mjchiu@ntu.edu.tw

Neurology® 2019;93:e1104-e1111. doi:10.1212/WNL.0000000000008088

NfL

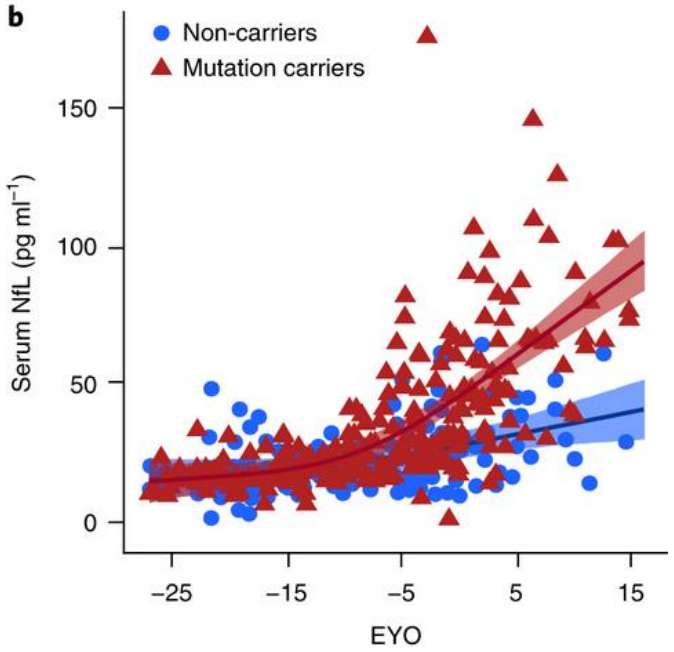
Figure 3 Motor and cognition progression in patients with PD with high or low plasma NfL levels in the follow-up study



Kaplan-Meier plots show outcomes for (A) motor progression and (B) cognitive progression in patients with Parkinson disease (PD) who had baseline neurofilament light chain (NfL) concentrations above or below the cutoff levels determined by receiver operating characteristic curve analyses.

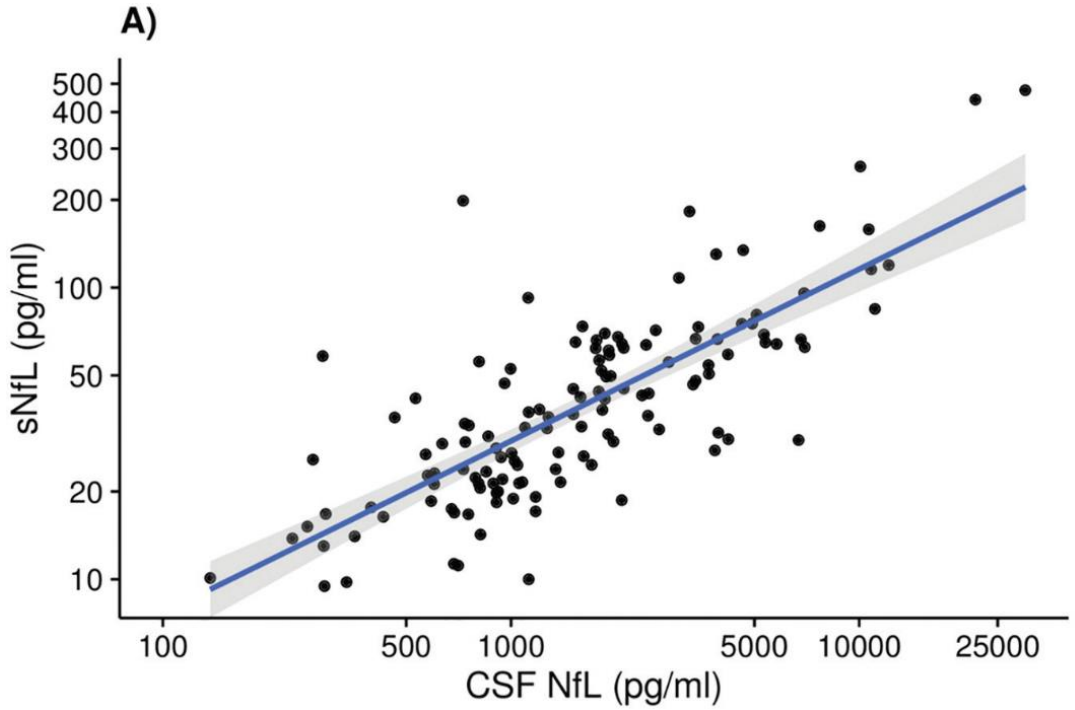
NFL in Alzheimer's diseases

- Study involving participants at 50% risk of carrying an autosomal-dominant Alzheimer's mutation in *APP, PSEN1* or *PSEN2* gene enrolled (DIAN cohort)
- Baseline serum (and CSF) NfL levels significantly increased for mutation carriers at -6.8 EYO (estimated years to onset)
- Rates of change in serum NfL conc. can discriminate mutation carriers from non-carriers as early as -16.2 EYO (nearly 10 years earlier than baseline serum NfL measurements alone)
- Changes in serum NfL can predict progression in familial Alzheimer's at early pre-symptomatic stages earlier than baseline NfL measurements alone



Preische et al Nature Med 2019

NFL IN MULTIPLE SCLEROSIS
Correlation between CSF and Serum/plasma levels in MS



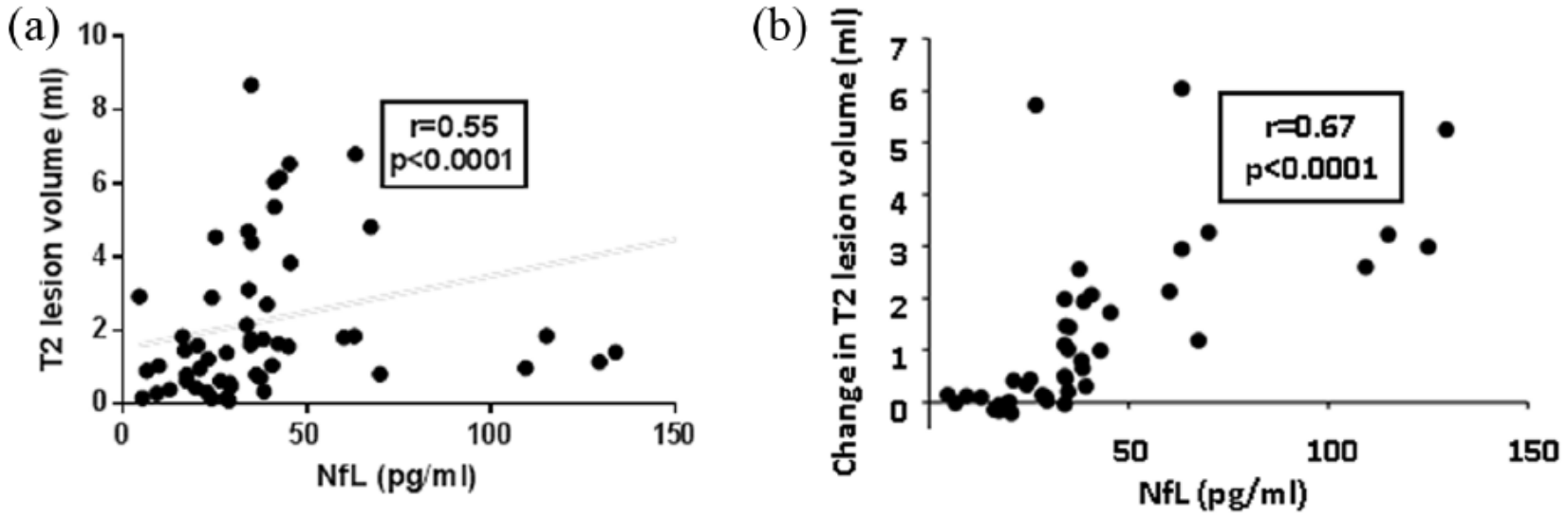
Di Santo et al. 2017

Levels in serum are about **40-100 fold lower** than in CSF

3. sNFL as prognostic biomarker

- Prognostic value at baseline for future relapses, new MRI lesions, brain volume loss and risk of disability worsening

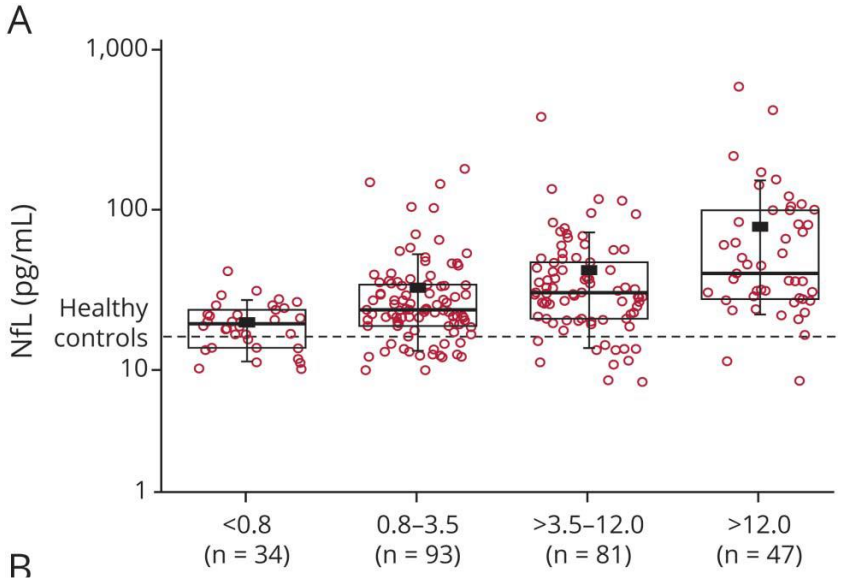
- Prognostic value in CIS patients later converting to MS

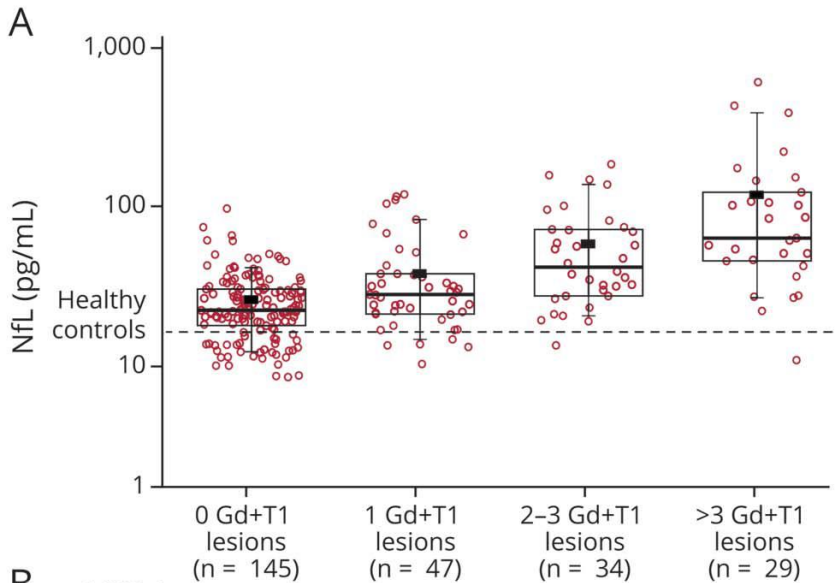


Baseline sNfL correlated significantly with T2 lesion volume and change in T2 lesion volume over time

Siller 2018

1. sNFL as biomarker of disease and MRI activity





Kuhle 2019

NfL concentrations increased gradually with higher baseline T2 lesion volume

NfL concentrations were higher in patients with Gd+ lesions compared with those free of Gd+ lesions

2. NFL as biomarker of treatment response

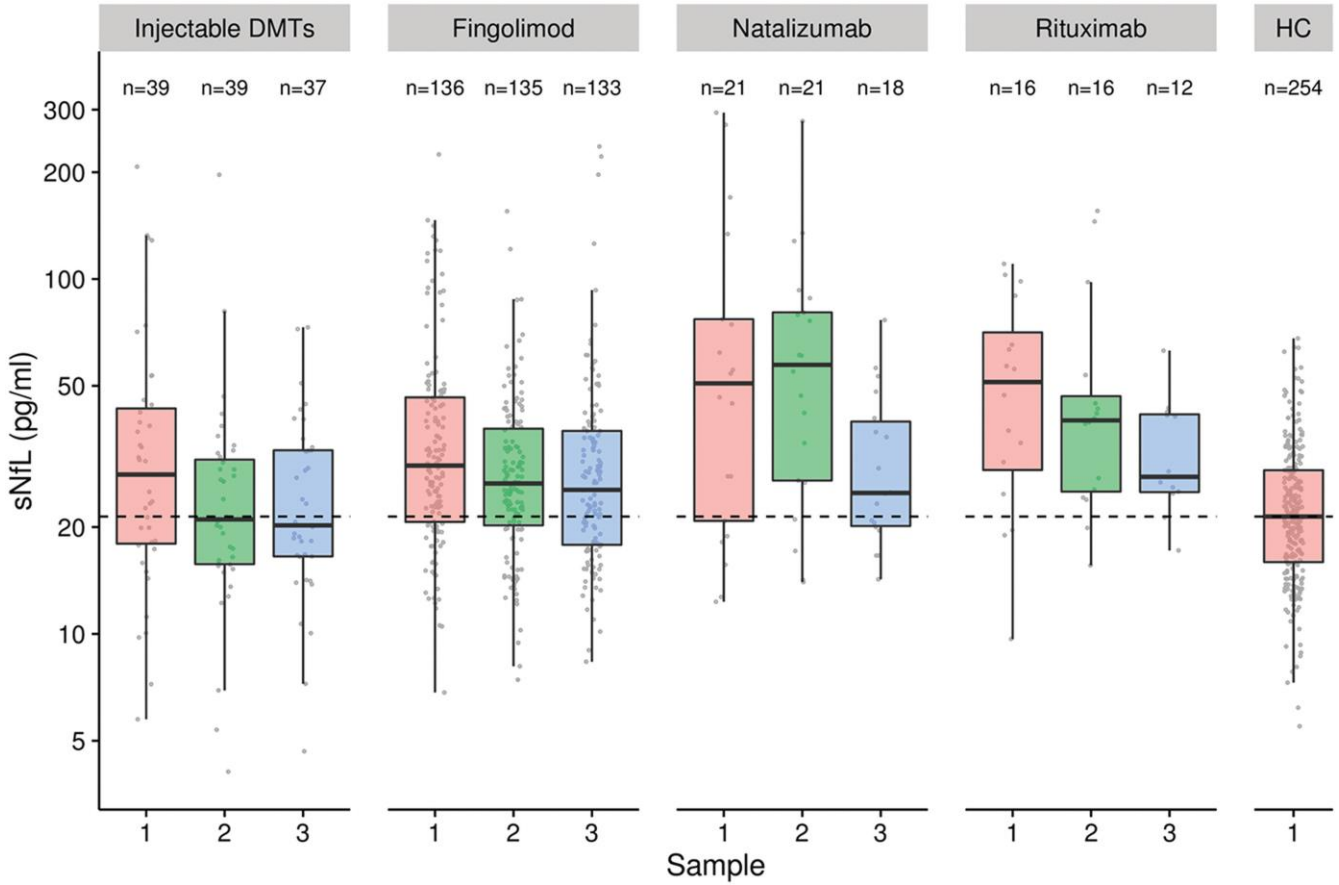
Inverse correlation was found between sNFL levels and treatments

TREATMENT	FOLLOW-UP	REF
β-interferons	6-12-24months	Siller 2018, Di Santo 2017, Novakova 2017, Varhaug 2018, Kuhle 2019
Glatiramer acetate	6-13 months	Siller 2018, Di Santo 2017, Novakova 2017
Dimethyl fumarate	13 months	Siller 2018
Teriflunomide	13 months	Siller 2018
Natalizumab	6-12 months	Di Santo 2017, Novakova 2017
Rituximab	6-12 months	Di Santo 2017, Novakova 2017
Alemtuzumab	12- Up to 102 months	Novakova 2017, Akgun 2019, Hyun 2019
Fingolimod	6-12 months	Di Santo 2017, Novakova 2017, Piehl 2017, Kuhle 2019

Effect of treatments on NFL levels

Association between time under treatment and sNfL during follow-up (T0, 6 months, 12 months)

sNfL levels decreased in patients starting injectable DMTs, fingolimod, natalizumab, or rituximab over time.



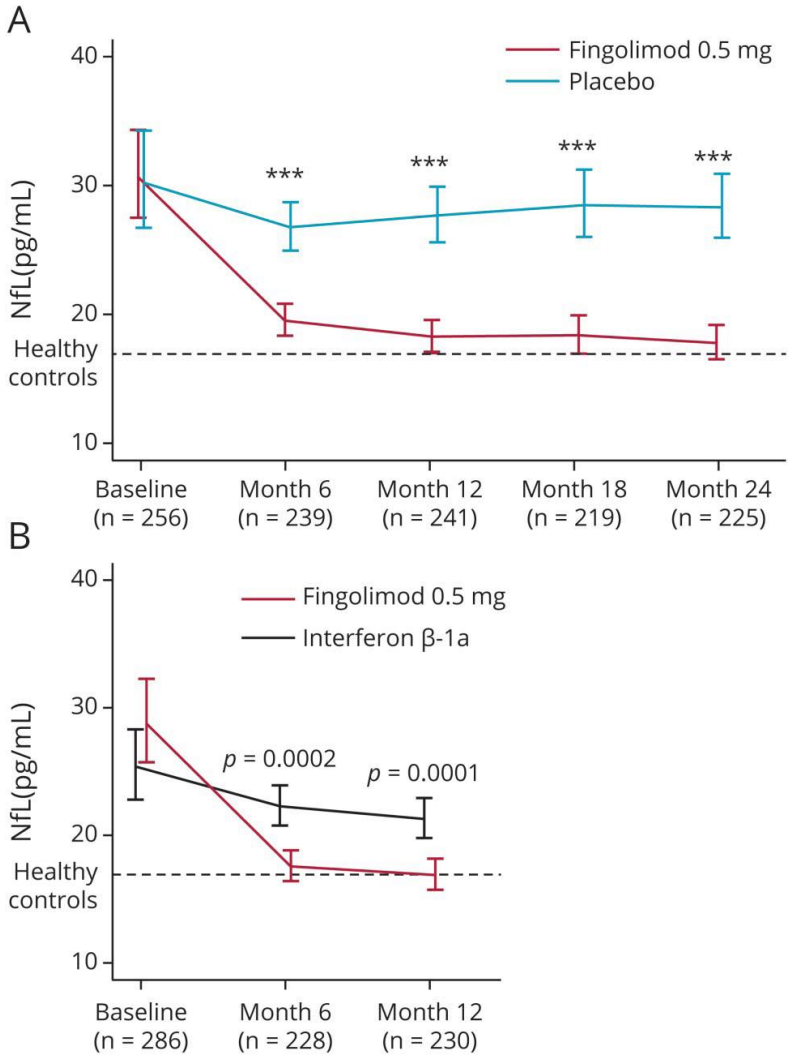
Di Santo 2017

sNFL levels in Fingolimod treatment

sNfL concentrations in the Fingolimod group were significantly lower compared with both placebo and IFN-β-1a.

FREEDOMS

TRANSFORMS



Kuhle 2019

JAMA Neurology | Original Investigation

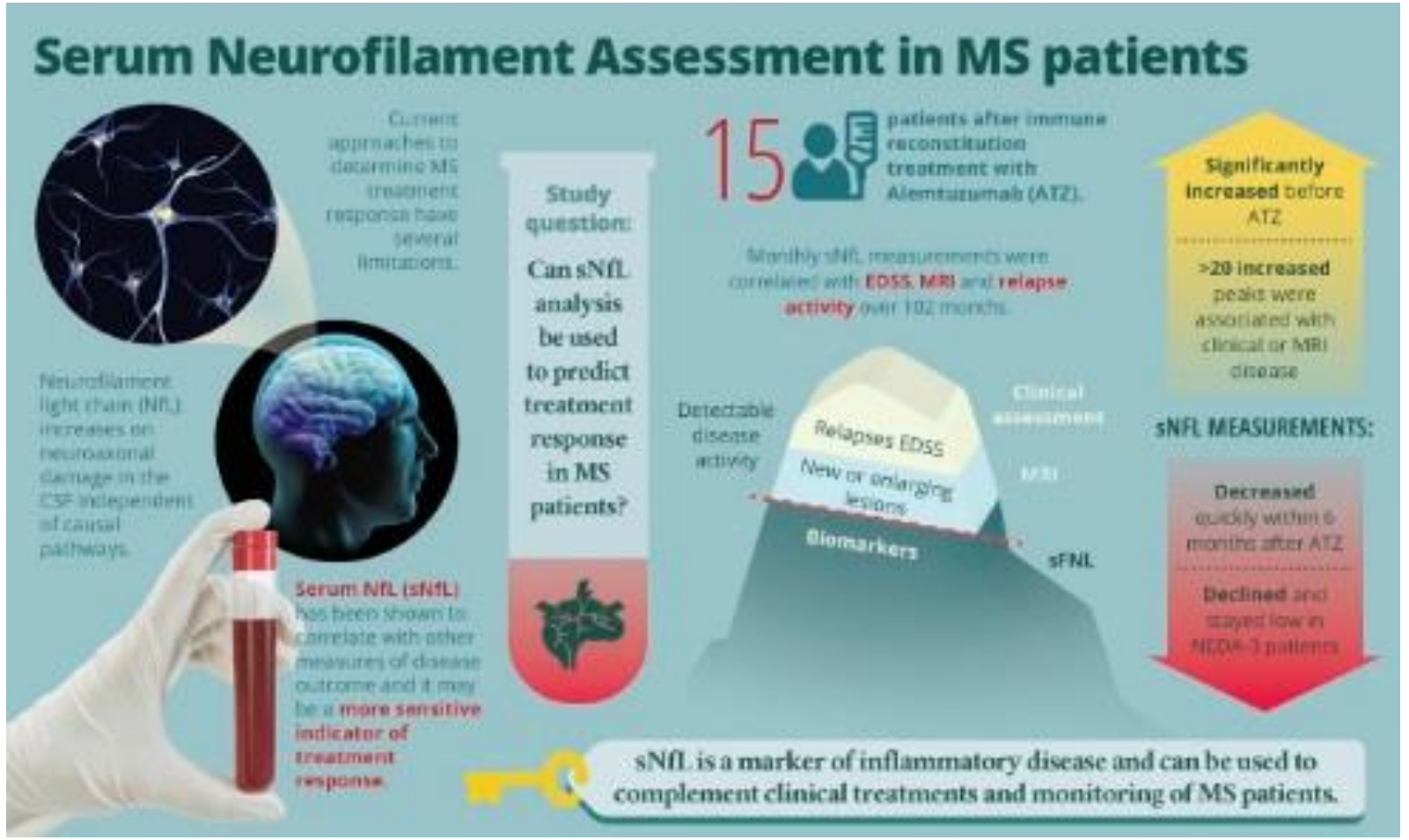
Serum Neurofilament Light Chain Levels in Patients With Presymptomatic Multiple Sclerosis

Kjetil Bjornevik, MD, PhD; Kassandra L. Munger, ScD; Marianna Cortese, MD, PhD; Christian Barro, MD; Brian C. Healy; David W. Niebuhr, MD; Ann I. Scher, PhD; Jens Kuhle, MD, PhD; Alberto Ascherio, MD, DrPH

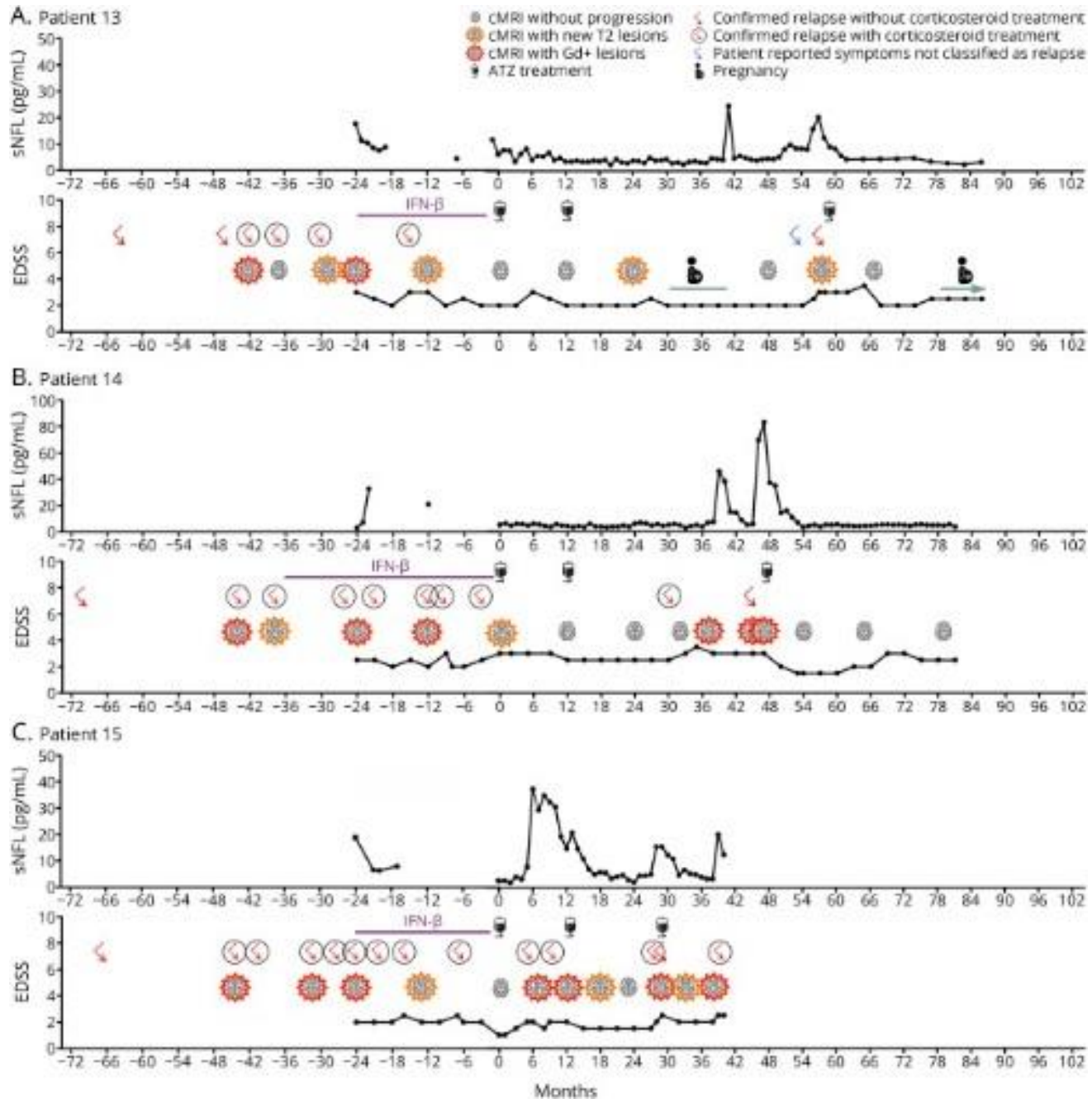
CONCLUSIONS AND RELEVANCE The levels of sNfL were increased 6 years before the clinical MS onset, indicating that MS may have a prodromal phase lasting several years and that neuroaxonal damage occurs already during this phase.

NFL IN INDIVIDUAL MONITORING

Profiling individual clinical responses by high-frequency serum neurofilament assessment in MS



Akgun K et al, Neurol, neuroimmunol, Neuroinfl 2019


A. Patient 13
B. Patient 14
C. Patient 15
cMRI without progression
cMRI with new T2 lesions
cMRI with Gd+ lesions
ATZ treatment
Confirmed relapse without corticosteroid treatment
Confirmed relapse with corticosteroid treatment
Patient reported symptoms not classified as relapse
Pregnancy
sNFL (pg/mL)
EDSS
IFN-β
Months

- After ATZ, sNfL decreased quickly within the first 6 months.
- In patients classified as NEDA-3, sNfL declined and persisted at an individual low steady-state level.
- Definition of clinically significant increase for each patient based on individual steady-state level
- 34 sNfL peaks with a >20 fold increase could be detected, which were associated with clinical or MRI disease activity, or even patient-reported relapse-suspicious symptoms
- sNfL started to increase earliest 5 months before, peaked at clinical onset, and recovered within 4–5 months.
- sNfL presented at higher levels in active patients requiring ATZ retreatment compared with responder patients.

SIMOA TECHNOLOGY AT CReSM

Il laboratorio del CReSM si è recentemente dotato dello strumento SR-X *Ultra-Sensitive Biomarker Detection System.*

- sistema compatto
- rilevamento a livelli di ultra-sensibilità di singoli biomarcatori o di diverse molecole in multiplex (fino a quattro biomarcatori per campione),
- volumi ridotti di campione (25 ul di siero/plasma per i NFL)
- ampio spettro di matrici biologiche

Lo strumenti SR-X, e la strumentazione SIMOA disponibili nel laboratorio del CReSM

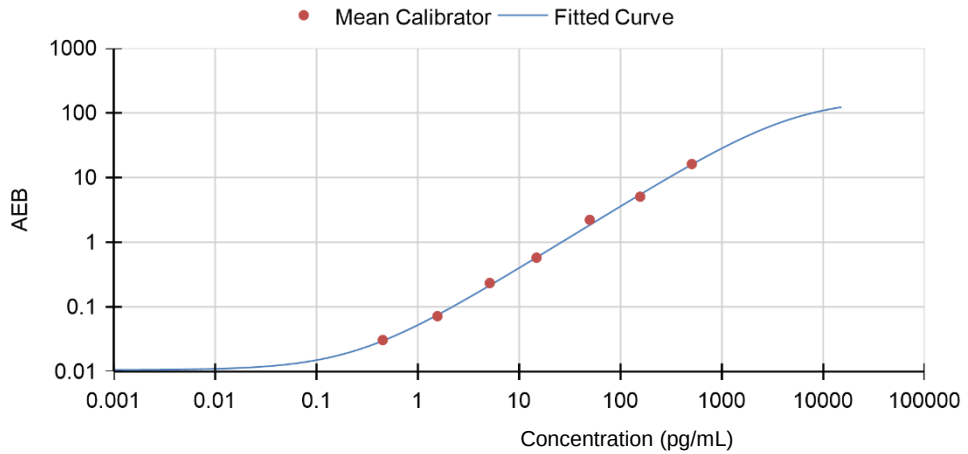
Esperimento #1 13/06/2019

Run Name	2019-06-13 Run 1
Run Date	6/13/2019
Instrument Serial #	1905QP0428
Software Version	1.0.4

For Research Use Only

Run Report Created: June 13, 2019

Plate Layout 2019-06-13_12-05 NF-light Advantage 20190613153309



Curve Information	
Fit Algorithm	4PL
Weighting Factor	WeightOverYSquared
R²	**0,997751474**
Date Created	June 13, 2019
Created by	LABUSER

Fit Coefficients	
A	1,06E-002
B	9,70E-001
C	5,07E+003
D	1,65E+002

Fit Equation

$$Y(x) = B + \frac{A - B}{1 + (\frac{x}{C})^D}$$

Real-life experience with NFL at CReSM

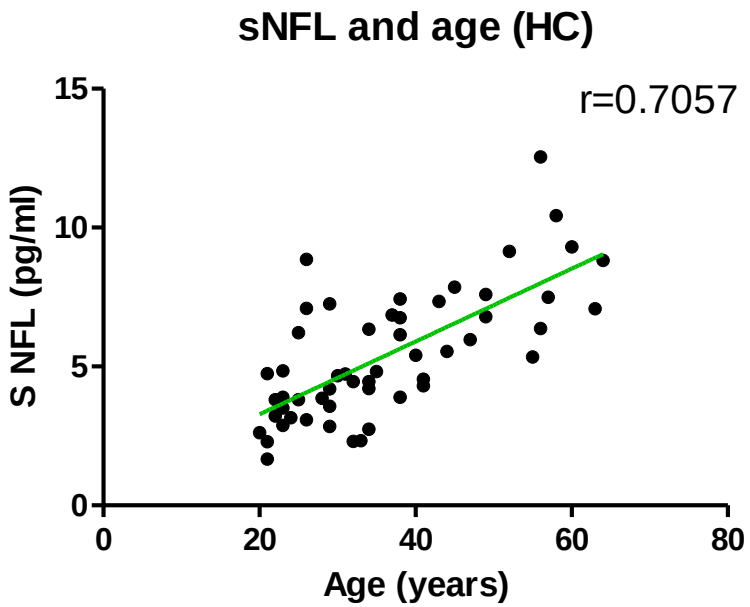
N= 24 NF-light SIMOA assay sessions

- 23 assays with kit 103186,
- 1 assay with new SR-X diluent (kit 103400)

N= 1058 samples from 897 participants (HC and patients)

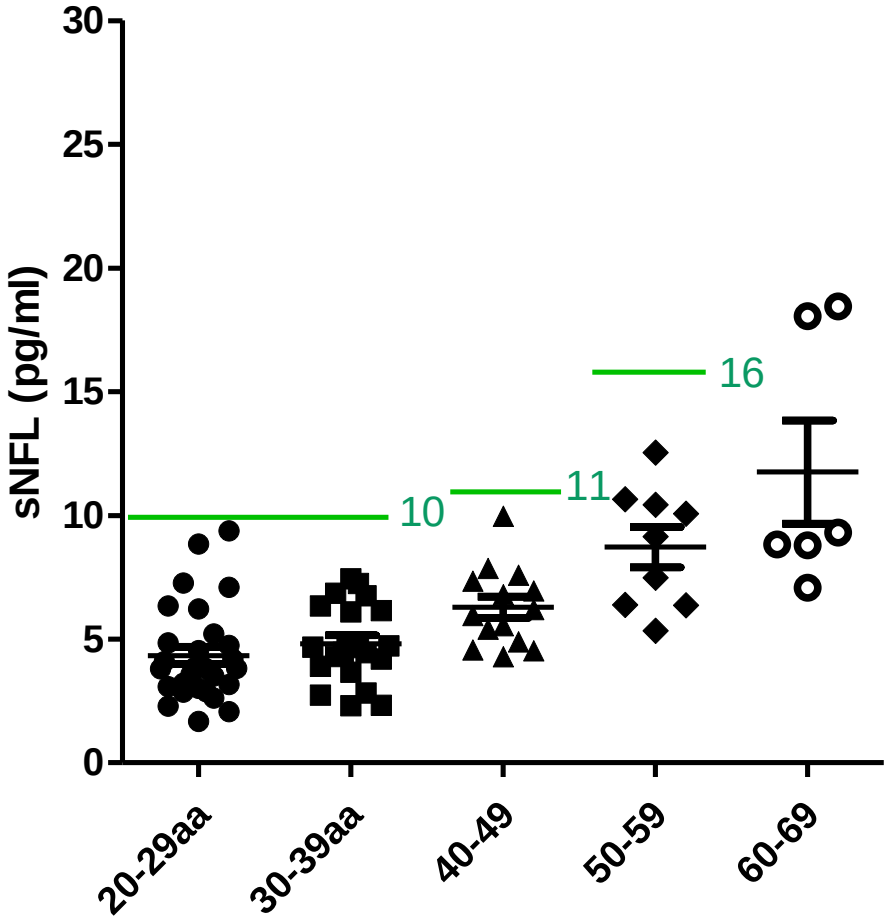
- 79 serum sample from HC
- 58 serum samples at lumbar puncture
- 58 CSF samples from patients at lumbar puncture
- 848 serum samples from MS patients pre-treatment or during follow-up
- 15 plasma samples from HC

NFL LEVELS in HC at CReSM



	20-29aa	30-39aa	40-49	50-59	60-69
Number of values	29	21	14	9	6
Minimum	1.668	2.31	4.303	5.35	7.081
25% Percentile	3.046	3.774	4.814	6.376	8.36
Median	3.81	4.664	6.086	9.144	9.065
75% Percentile	5.029	6.242	7.406	10.55	18.15
Maximum	9.366	7.435	9.969	12.54	18.45
Mean	4.338	4.807	6.283	8.715	11.75
Std. Deviation	1.918	1.587	1.589	2.428	5.094
mean +2sd	8.174	7.981	9.461	13.571	21.938
mean +3sd	10.092	9.568	11.05	15.999	27.032

CUT-OFF definition



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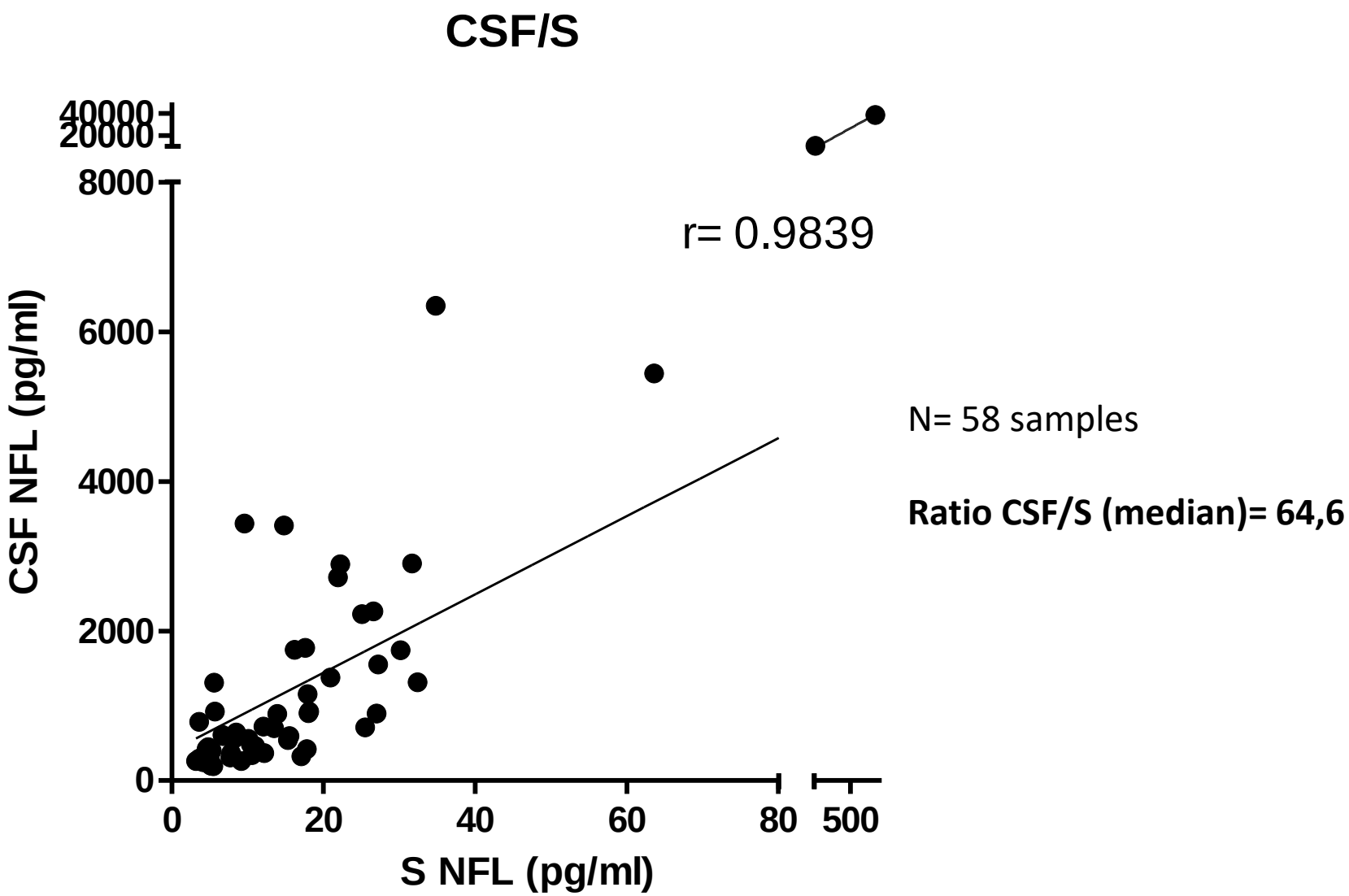
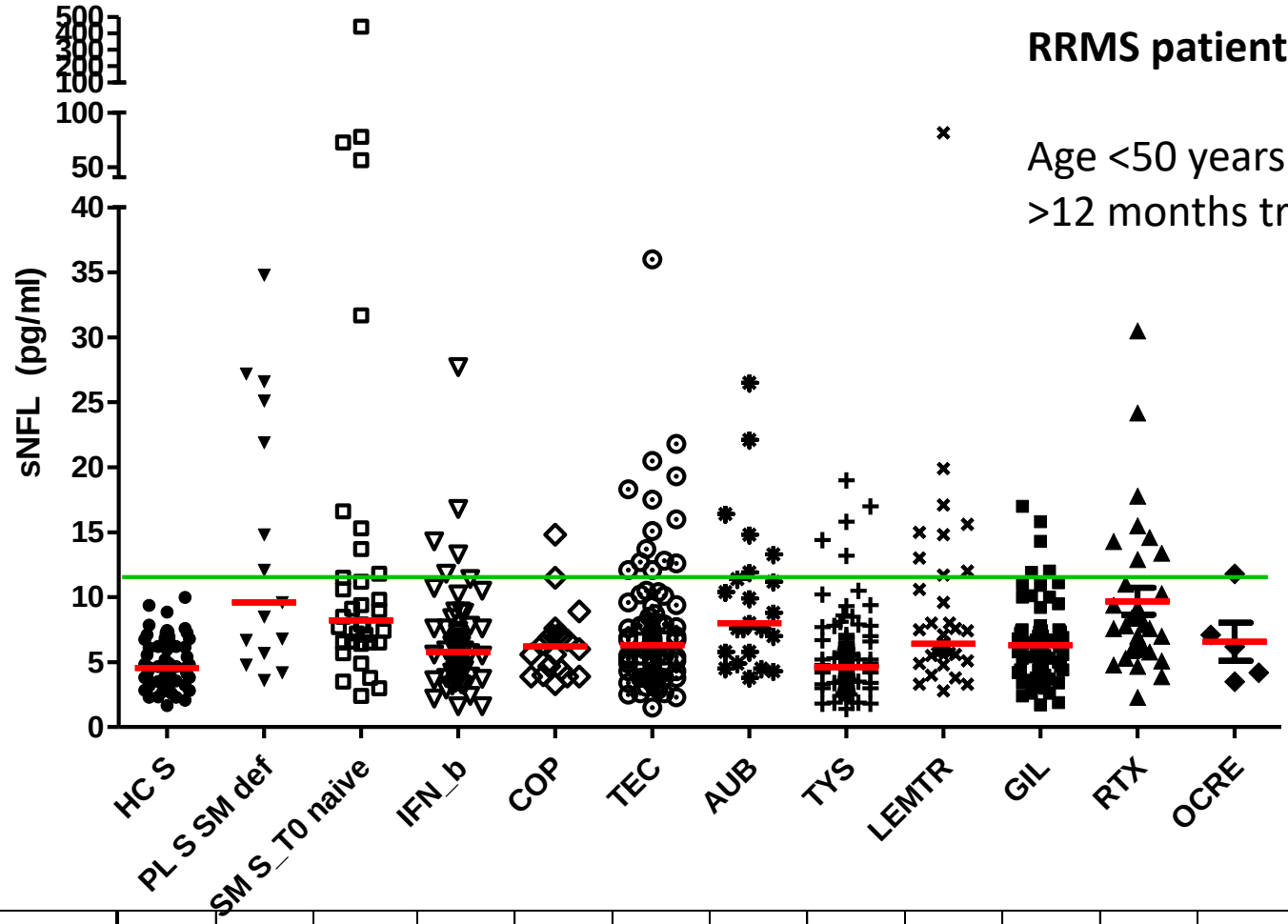
NFL LEVELS in patients:
correlation between serum and CSF NFL levels



Effect of treatments in MS patients

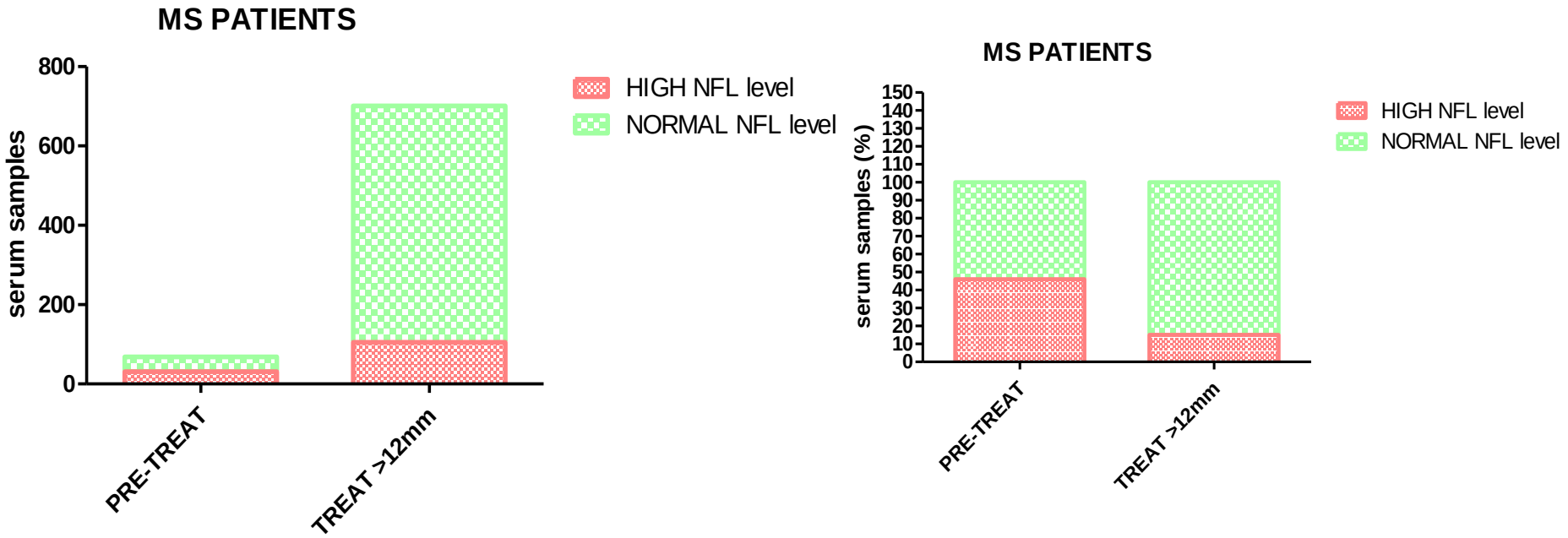


RRMS patients (n=583)

Age <50 years old
>12 months treated

<50 years old	HC S	PL S SM def	SM S_T0 naive	IFN_b	COP	TEC	AUB	TYS	LEMTR	GIL	RTX	OCRE
Number of values	64	15	34	58	20	90	23	93	37	74	32	5
Minimum	1.668	3.6	2.4	1.6	3.3	1.5	3.8	1.4	2.8	1.7	2.3	3.5
Median	4.56	9.6	8.2	5.8	6.2	6.3	8	4.6	6.4	6.3	7.85	6.2
Maximum	9.969	34.8	441.5	27.7	14.8	36	26.5	19	81.4	17	30.5	11.8
Mean	4.918	14.16	26.99	6.681	6.46	7.52	9.917	5.41	10.01	6.57	9.684	6.56
Std. Deviation	1.879	10.24	75.47	4.198	2.793	5.191	5.741	3.23	12.76	3.011	5.938	3.272

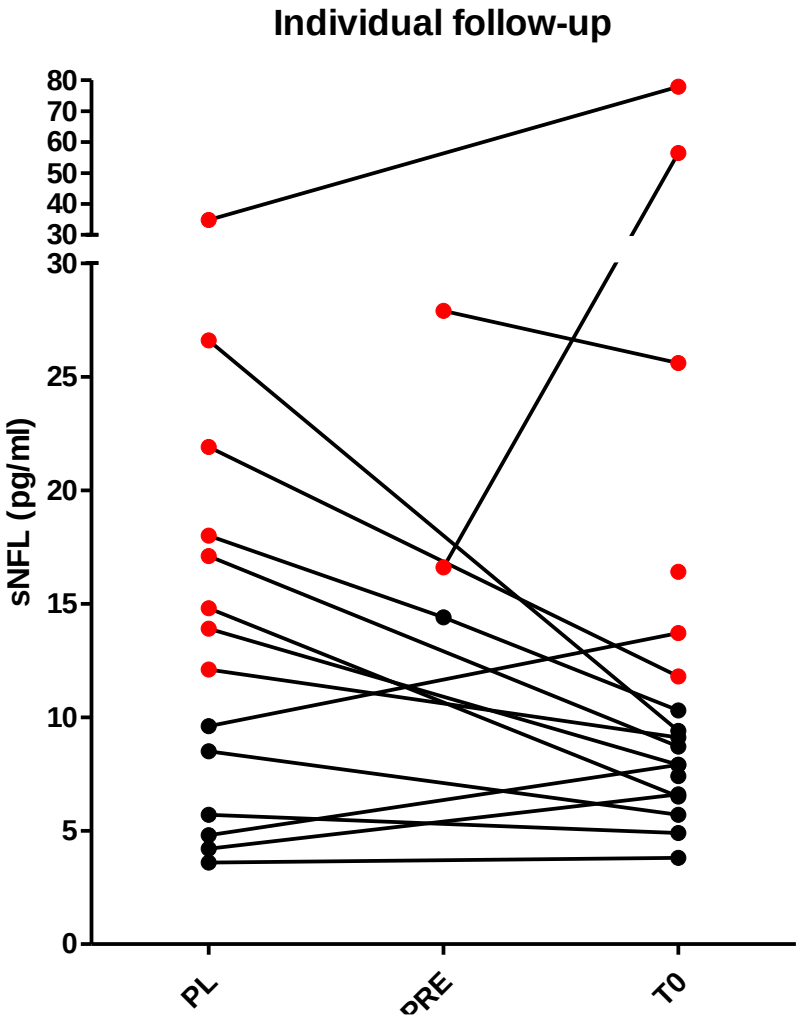
Prevalence of NFL levels in naive and treated MS patients



MS patients	high NFL		low NFL		tot
naive	31	(46%)	37	(54%)	68
Treat>12mm	105	(15%)	596	(84%)	701

NFL LEVELS in patients : individual follow-up

Pre-treatment follow-up in MS patients



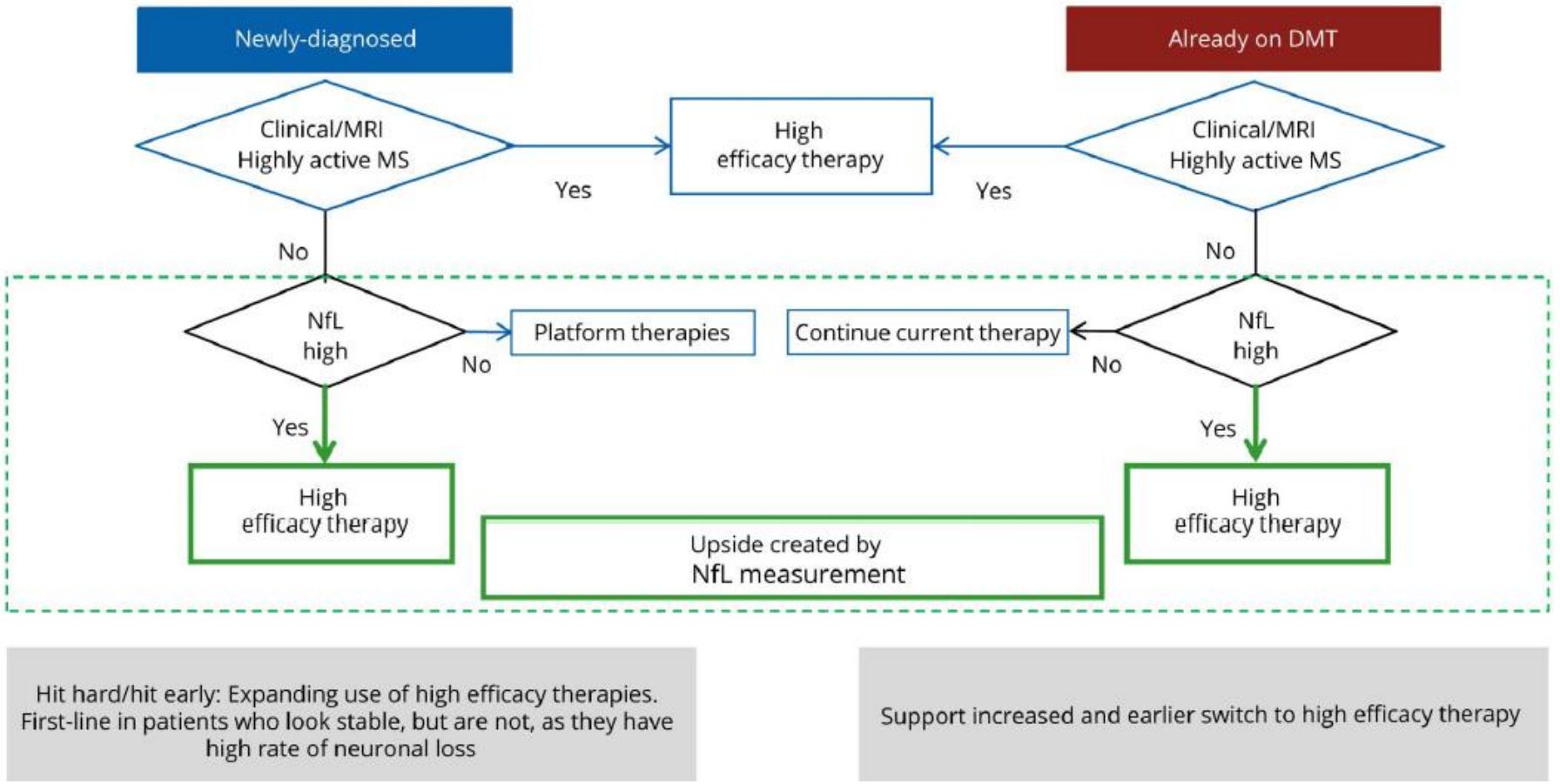
IMPLEMENTING NFL IN CLINICAL PRACTICE

NEED OF ASSAY STANDARDIZATION AND VALIDATION

- Prospective studies
- Larger cohorts
- Baseline+follow-up data
- External calibration of the assay
- Compare results between different centers
- Determining cut-off values
  - Cut-off based on healthy people values (age-dependent)
  - Intra-individual cut-off value
- Defining the best time-interval to monitor NFL levels

Once validated, replicated, standardized, and widely accessible, blood NfL could be a game changer in clinical neurology, a **simple blood test to monitor axonal injury**, which should help neurologists to select and guide the choice of disease-modifying treatments, which are becoming available for many neurologic diseases.

Proposed flow-chart for use of NFL for individual therapeutic decision making in MS



Leppert and Kuhle 2019

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