sNFL as a biomarker for routine management of MS patients: the role of a disease-based biobank.

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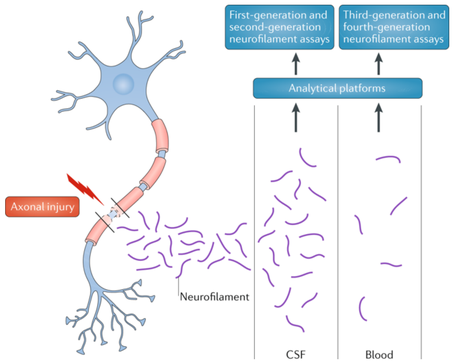
Abstract

INTRODUCTION: Serum Neurofilament Light Chain (sNFL) is the most promising biomarker of disease activity and treatment efficacy in multiple sclerosis (MS). However its utilization for routine individual patient care is still to be demonstrated.
MATERIAL AND METHODS: Samples and clinical data for this study have been collected within our MS Biobank, that supports MS scientist in their research projects. sNFL were measured by SIMOA assay in 79 HCs and in 961 MS patients (n=1130 samples). sNFL were cross-sectionally evaluated in MS patients at different disease stages including diagnostic time, immediately before treatment, and during treatment with the main disease modifying treatments (DMTs).Clinical assessment was performed to evaluate correlations between sNFL, MRI and relapses.
RESULTS: sNFL cut-off values were defined testing HCs. Progressive MS patients showed higher sNF-L levels and a greater prevalence of high sNFL levels (32%) compared to relapsing MS patients (16%). Patients experiencing MRI and/or clinical activity close to sNFL dosage (+/-60 days) showed higher levels than stable patients; high NFL levels were observed in a substantial percentage of MRI active patients (72%) and clinically active patients (75%). All DMTs lower sNFL in RRMS patients treated for more than 12 months compared to untreated patients; though, 12% of treated patients still showed high NFL levels.
DISCUSSION AND CONCLUSIONS: This study provides a real-life picture of sNFL in a large cohort of MS patients, showing that implementation of sNFL in everyday clinical practice could be revolutionary in the routine management of MS patients. The availability of a structured MS biobank improved the conduction of this large study, allowing: 1) fast recruitment of patients and organized collection of Samples and data, according to the Procedure of the biobank. 2) availability of samples and data to further studies raising from this one, including retrospective studies on the same patients.

Background

sNFL as promising biomarker in MS



Neurofilaments are neuronal structural proteins which are released in CSF and, in lower concentrations, in serum upon axonal damage of any kind.

Simoa technology enabled **serum NFL (sNFL)** quantification, which is a less invasive test allowing repeated measures during patients' follow-up.

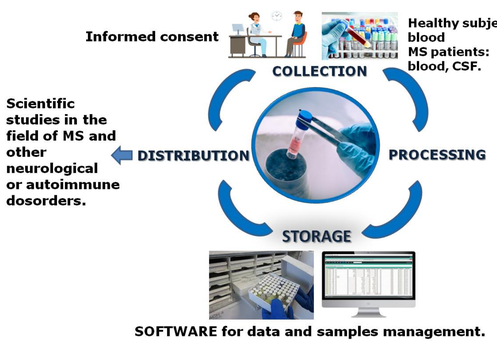
Now, serum Neurofilament Light Chain (sNFL) is the most promising biomarker of disease activity and treatment efficacy in multiple sclerosis (MS).
However its use for routine individual patient care is still to be demonstrated.

Quantification of "normal values"

The CRESM Biobank: BB-CRESM

The **CRESM Biobank** is an institutional structured biobank that works for the collection, storage and distribution of biological samples and associated data to support quality research in Multiple Sclerosis and other neurological and autoimmune diseases.

BB-CRESM STRUCTURE



Aliquots of serum, plasma, CSF. PBMCs isolation. RNA extraction.

BB-CRESM IN NUMBERS

1269	PARTECIPANTS: 62 HEALTHY CONTROLS, 1086 MS PATIENTS , 121 PATIENTS AFFECTED BY OTHER NEUROLOGICAL DISORDERS
3086	BLOOD WITHDRAWALS (at diagnosis and during follow up)
549	PAIRED CSF AND BLOOD SAMPLES AT DIAGNOSIS (40 ml blood, 15 ml CSF)
2948	SERUM SAMPLES
2932	PLASMA SAMPLES
2933	DNA SAMPLES
2965	WHOLE BLOOD RNA SAMPLES
2794	PBMCs STORED using RNA stabilyzer

Methods

Serum samples for sNFL studies were selected from **CRESM Biobank.** in particular, samples from MS patients were prospectively collected between Apr-2019 and Jan-2020.

Healthy participants

Inclusion criteria:
- Absence of neurological or autoimmune disease and family history
- Age 18-70 years

Healthy participants n=79
- Median age: 35 years (range 20-68 years)
- Female/male: 62/79 (78.5%)

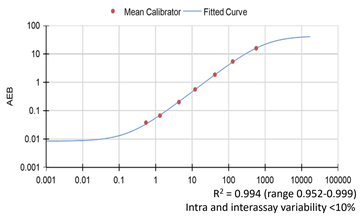
CUT-OFF DEFINITION

MS patients

Inclusion criteria:
- Diagnosis according to Mc Donalds criteria
- Age 18-70 years

MS patients n=961 (n=1130 samples)

- **MS types**: RRMS n=830 patients; PPMS n=53 patients; SPMS n=78 patients
- **Disease stage**: Diagnosis/Naïve n=78 patients; DMT during f-up n=823 patients; DMT switch n=47 patients; No therapy n=13 patients
- **Disease phase**: Disease activity* (n= 38 patients); NEDA-3* >12 months (n=518 patients)



Semi-automatic SR-X *Ultra-Sensitive Biomarker Detection System*

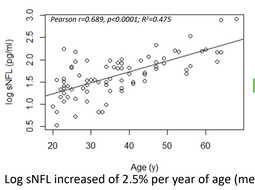
We carried out **34 NF-light SIMOA assay sessions** with optimal standard curves.

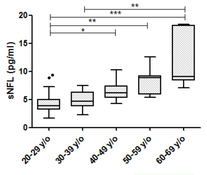
Results are reliable

ADDITIONAL SERUM SAMPLES FROM 20 HCs AND FROM 961 MS PATIENTS ARE CURRENTLY PROSPECTIVELY COLLECTED EVERY 6 MONTHS IN THE BB-CRESM FOR FURTHER sNFL STUDIES.

Results

Age-related cut-off definition from HC samples



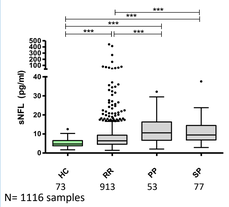


Log sNFL increased of 2.5% per year of age (mean)

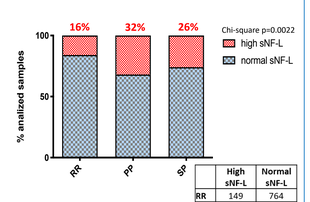
Cut-offs were used to distinguish **high/pathological** (over cut-off) from **normal** values in MS patients

Age	N	Mean sNFL	CUT-OFF =mean+3SD
20-29	30	4,4	10,0
30-39	21	4,9	9,6
40-49	15	6,3	11,0
50-59	7	8,2	15,7
60-69	6	11,7	27,0

Progressive patients demonstrate higher sNFL and a higher prevalence of high sNFL

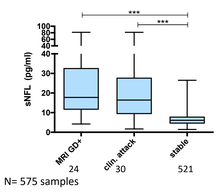


N= 1116 samples

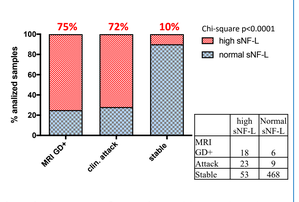


	High sNF-L	Normal sNF-L
RR	149	764
PP	17	36
SP	20	57

Radiologically/clinically active patients demonstrate higher sNFL and a higher prevalence of high sNFL



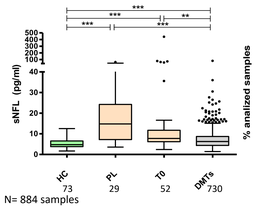
N= 575 samples



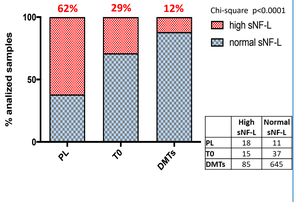
	high sNF-L	Normal sNF-L
MRI GD+	18	6
Attack	23	9
Stable	53	468

Clinical analysis was performed on 700 RRMS patients
Clinical activity range: 60 days prior or after MRI and/or clinical attack.

DMTs reduce sNFL levels relative to untreated patients



N= 884 samples



	High sNF-L	Normal sNF-L
PL	18	11
TO	15	37
DMTs	85	645

We considered RRMS patients treated for more than 12 months.
Despite evident DMTs efficacy, **12% of treated patients still showed high sNFL levels.**

Conclusions

sNF-L may be a revolutionary monitoring and treatment decision biomarker in everyday clinical practice

ROLE OF THE BB-CRESM IN SUPPORTING sNFL STUDIES AT CRESM: i) Support in the organization of a structured capillary collection of samples and associated data; ii) ensure the collection, processing and storage of samples and data following rigorous quality procedures; iii) promote the distribution of samples already provided with sNFL quantification; iv) provide a large amount of samples for further studies (even retrospective studies).

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