Longitudinal and prospective evaluation of serum neurofilament light chain in acute ischemic stroke patients by Single Molecular Array technology

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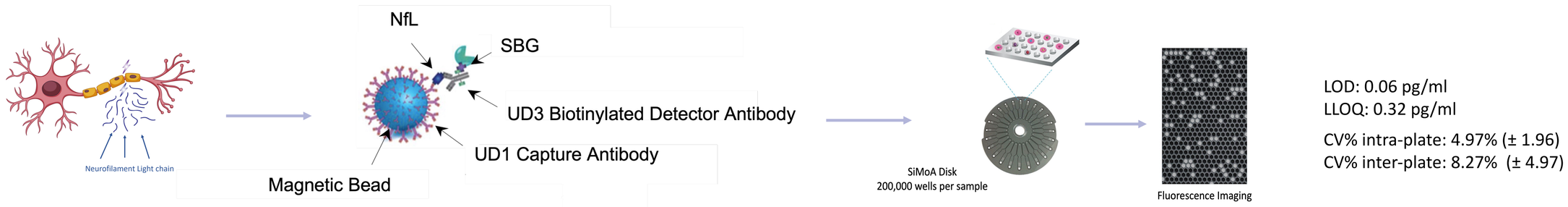
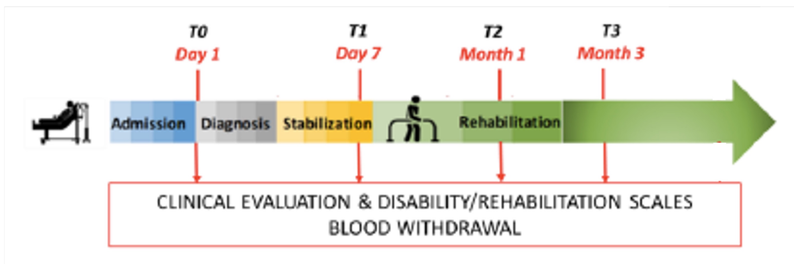
Background and Methods

Neurofilament Light Chain (NfL) are intermediate filament proteins released by neurons upon brain injury. They have attracted attention as ischemic stroke biomarkers; however, their quantification through traditional approaches is difficult due to their low concentration in peripheral blood. To overcome this limitation, we used **Single Molecule Array** (SiMoA™, Quanterix) technology, a new ultrasensitive digital ELISA able to isolate and quantify single protein targets present in the biological fluids down to femtomolar concentrations.
Serum samples are incubated with UD3 biotinylated detector antibody and with paramagnetic beads coupled with UD1 capture antibody. The formed immunocomplex is isolated from the biofluid by magnetic separation. The system can dispose each microbead in a single femtoliter-sized well together with resorufin ß-D-galactopyranoside (RGP) substrate and with streptavidin ß-galactosidase (SBG) enzyme to ensure a high local concentration of fluorescent signal and detection of the target as single molecule.



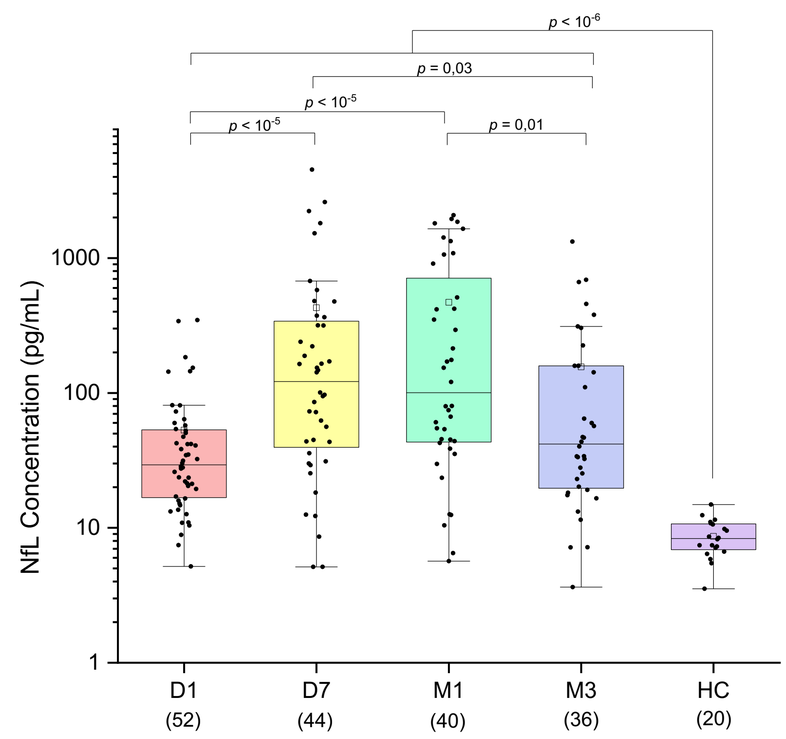
Study design and Aim



The aim of this longitudinal and prospective study is the evaluation of serum concentration of neurofilaments (sNfL) released from the neurovascular unit in patients with ischemic stroke (protocol n. 2250 coordinated by ICSM, Pavia, Italy).
54 patients with stroke (50% female, 50% male, age 73.1 ± 12.6 years) were enrolled and compared with 20 healthy subjects (55% female, 45% male, age 48.2 ± 9.6 years) used as controls. Time-points of evaluation were at D1 (within 24 hours from symptoms onset), D7 (day 7), M1 (day 30 ± 3) and M3 (day 90 ± 5) after stroke. At each time-points, clinical and rehabilitation scales were assessed to correlate the temporal profile of sNfL with patients' outcome.

1. Temporal profile of serum NfL

Levels of sNfL were higher in stroke vs. HC and showed a maximum peak at D7. At time point M3 sNfL decreased significantly, nevertheless remaining higher than sNfL levels measured in HC. In the acute phase (D1) sNfL levels were significant associated with neurological deficit ($r = 0.31$, $p = 0.03$) and with the disability before the event ($r = 0.47$, $p = 4.38\ 10^{-4}$).



2. Correlation between NfL and clinical/rehabilitation scales

A correlation analysis was performed between sNfL and the following clinical/rehabilitation scales: National Institute of Stroke Severity Scale (**NIHSS**) for stroke severity, modified Rankin Scale (**mRS**) for functional outcome, Trunk Control Test (**TCT**) and Functional Ambulation Classification (**FAC**) for motor deficit, Functional Independence Measure (**FIM**) for a more comprehensive evaluation of patients' disability in activities of daily living.
Results show a longitudinal correlation between sNfL levels and the scales for each time-points.

		Spearman's rho	*p-value*
NIHSS	D7	0,65	$2,21 \times 10^{-6}$
	M1	0,68	$1,49 \times 10^{-6}$
	M3	0,49	0,003
mRS	D7	0,60	$2,34 \times 10^{-5}$
	M1	0,62	$2,31 \times 10^{-5}$
	M3	0,40	0,03
TCT	D7	-0,60	$2,10 \times 10^{-5}$
	M1	-0,65	$6,11 \times 10^{-6}$
	M3	-0,48	0,004
FAC	D7	-0,63	$5,32 \times 10^{-6}$
	M1	-0,61	$2,48 \times 10^{-5}$
	M3	-0,58	$3,61 \times 10^{-4}$
Total FIM	D7	-0,74	$1,79 \times 10^{-8}$
	M1	-0,71	$2,64 \times 10^{-7}$
	M3	-0,66	$2,14 \times 10^{-5}$
Motor FIM	D7	-0,66	$1,57 \times 10^{-6}$
	M1	-0,69	$1,05 \times 10^{-6}$
	M3	-0,55	$6,88 \times 10^{-4}$
Cognitive FIM	D7	-0,73	$2,32 \times 10^{-8}$
	M1	-0,63	$1,52 \times 10^{-5}$
	M3	-0,56	$5,58 \times 10^{-4}$

3. Prognostic role of NfL in ischemic stroke

To assess the capability of sNfL to predict long-term patients' outcome a prospective correlation analysis was performed taking into account the levels of sNfL measured at their maximum peak of blood release *i.e.* D7.
A significant correlation was found with both motor and cognitive parameters displaying a potential relevance of sNfL protein in predicting patients' recovery.

		Spearman's rho	*p-value*
NIHSS	D7 NfL M1	0,61	$5,62 \times 10^{-5}$
	D7 NfL M3	0,53	0,002
mRS	D7 NfL M1	0,55	$3,66 \times 10^{-4}$
	D7 NfL M3	0,37	0,04
TCT	D7 NfL M1	-0,51	$9,80 \times 10^{-4}$
	D7 NfL M3	-0,44	0,02
FAC	D7 NfL M1	-0,55	$3,66 \times 10^{-4}$
	D7 NfL M3	-0,57	$9,52 \times 10^{-4}$
Total FIM	D7 NfL M1	-0,62	$2,86 \times 10^{-5}$
	D7 NfL M3	-0,65	$9,23 \times 10^{-5}$
Motor FIM	D7 NfL M1	-0,58	$1,26 \times 10^{-4}$
	D7 NfL M3	-0,51	0,004
Cognitive FIM	D7 NfL M1	-0,56	$2,27 \times 10^{-4}$
	D7 NfL M3	-0,57	0,001

Conclusions

SiMoA™ was able to measure sNfL with high accuracy after acute ischemic stroke. Results show the longitudinal and prospective relevance of sNfL as biomarker of brain injury and recovery thanks to their correlation with stroke severity and patients' functional outcome assessed through specific motor and cognitive disability scales over a 3-month follow-up.

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