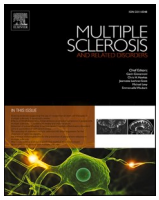
Multiple Sclerosis and Related Disorders 82 (2024) 105412

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard



Original article

Serum and cerebrospinal fluid neurofilament light chains measured by SIMOA™, Ella™, and Lumipulse™ in multiple sclerosis naïve patients.

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ARTICLE INFO

Keywords:
Neurofilament light chains
Multiple sclerosis
Serum and cerebrospinal fluid
Platforms
Assays

ABSTRACT

Background: Neurofilament light chains (NfL) are cytoskeletal biomarkers of axonal damage, about 40-fold higher in cerebrospinal fluid (CSF) compared to serum, and requiring ultrasensitive techniques to be measured in this latter fluid.
Objectives: To compare CSF and serum NfL levels in multiple sclerosis (MS) patients using different platforms.
Methods: 60 newly diagnosed relapsing-remitting MS patients (38 females; median age: 36.5 years, range: 15–60) were enrolled before steroid or disease-modifying treatments. CSF and serum NfL were measured with: the commercial Ella™ microfluidic platform (Bio-Techne), the Lumipulse™ Chemiluminescent Enzyme Immuno-Assay (Fujirebio), and the SIMOA™ on the SR-X instrument using NF-light assays (Quanterix).
Results: CSF and serum NfL absolute levels strongly correlated between assays, although being more elevated with Ella™. Passing-Bablok regression showed high agreement in measuring CSF NfL between assays (with greater proportional difference using Ella™), and very high agreement for serum comparing SIMOA™ and Lumipulse™. Similarly, the Bland-Altman comparison evidenced lower biases for Lumipulse™ for both fluids.
Conclusions: CSF and serum NfL in naïve MS patients are reliably measured with all assays. Although not interchangeable, SIMOA™ and Lumipulse™ showed high agreement for serum and CSF values.

1. Introduction

Neurofilament light chains (NfL) are cytoskeletal biomarkers of axonal damage, released into the cerebrospinal fluid (CSF) and subsequently measurable in peripheral blood (Teunissen and Khalil, 2012; Bittner et al., 2021). NfL have been extensively studied in association with several neurological disorders (Verde et al., 2019; Katzeff et al., 2022; Steinacker et al., 2016; Mariotto et al., 2018; Mattsson et al., 2010), including multiple sclerosis (MS) (Lycke et al., 1998), as a potential not disease-specific biomarker of neurodegeneration. In demyelinating diseases, NfL levels could predict conversion from radiologically or clinically isolated syndrome to clinically definite MS (Lycke et al., 1998), correlate with disability (Disanto et al., 2017; Håkansson et al., 2018) and may predict disease worsening (Ferreira-Atuesta et al., 2021; Barro et al., 2018), and relate with treatment response in bout-onset and progressive MS (Håkansson et al., 2017; Gunnarsson et al., 2011; Novakova et al., 2017; Piehl et al., 2018; Varhaug et al., 2017; Delcoigne et al., 2020).

To obtain repetitive measures over time, serum samples are minimally invasive if compared to CSF collection through a lumbar puncture, but absolute NfL levels significantly differ among fluids (Valentino et al., 2021; Sejbaek et al., 2019). In fact, NfL values are about 40-fold higher in serum (Disanto et al., 2017), requiring ultrasensitive techniques to be detected. The single-molecule assay (SIMOA™) is considered the gold standard in sensitivity to measure blood NfL (Revendova et al., 2022; Gauthier et al., 2021). It is a digital immunoassay based on two highly specific non-competing monoclonal antibodies that can detect single molecules bound to paramagnetic beads, reaching a sensitivity down to

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https://doi.org/10.1016/j.msard.2023.105412
Received 19 December 2023; Accepted 25 December 2023
Available online 26 December 2023

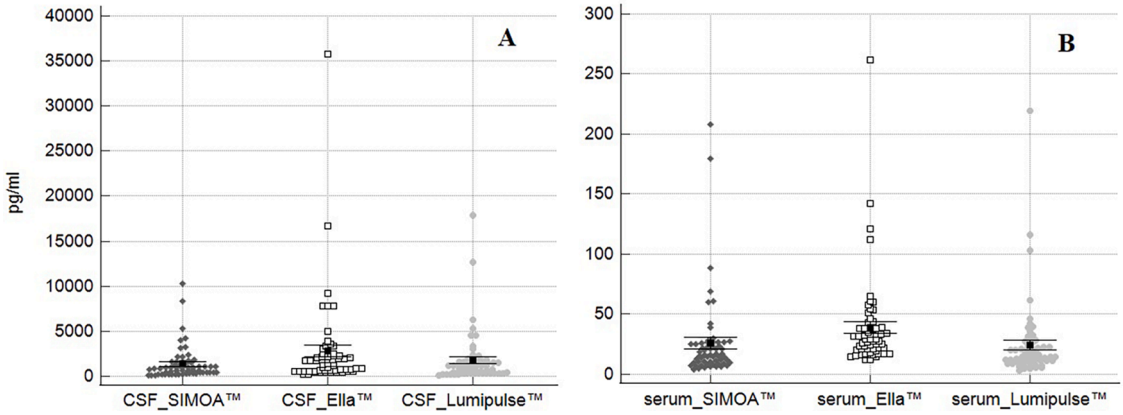

Fig. 1. CSF (A) and serum (B) NfL levels (pg/ml) measured by SIMOA™, Ella™, and Lumipulse™ in naïve MS patients. Each point represents an individual case. Black points and horizontal bars represent the medians and the 25th and 75th percentiles, respectively.

Table 1
NfL measurements in CSF and serum samples using the three platforms.

CSF Platform	Median (pg/mL)	Interquartile range (pg/mL)
SIMOA™	861.6	375.3–1533.4
Ella™	1590.5	596.0–2491.0
Lumipulse™	1105.0	363.0–1692.5
Serum Platform	**Median (pg/mL)**	**Interquartile range (pg/mL)**
SIMOA™	16.3	9.6–26.3
Ella™	29.3	20.8–38.7
Lumipulse™	14.7	10.6–25.5

Table 2
Spearman's rank correlation coefficient between SIMOA™, Ella™, and Lumipulse™ in naïve MS patients ($p<0.0001$ for all comparisons).

Correlation between SIMOA™ and either Ella™ or Lumipulse™		
	SIMOA™ versus:	
	Ella™ (95% CI)	Lumipulse™ (95% CI)
Serum NfL	0.93 (0.89–0.96)	0.92 (0.86–0.95)
CSF Nfl	0.93 (0.89–0.96)	0.95 (0.91–0.97)

Table 3
Passing-Bablok regression between SIMOA™, Ella™, and Lumipulse™.

Comparison between assays	Passing- Bablok		
	Intercept (95% CI)	Slope (95% CI)	Linear model validity (p)*
CSF			
SIMOA™/ Ella™	6.6 (−67.6 to 61.5)°	0.5 (0.5 to 0.6)	0.22
SIMOA™/ Lumipulse™	24.0 (−37.9 to 86.0)°	0.8 (0.7 to 0.9)	0.56
Lumipulse™/ Ella™	−35.5 (−56.0 to −18.8)	0.7 (0.7 to 0.7)	0.56
Serum			
SIMOA™/ Ella™	−5.8 (−7.4 to −3.5)	0.8 (0.7 to 0.9)	0.95
SIMOA™/ Lumipulse™	−0.04 (−2.1 to 1.4)°	1.0 (0.9 to 1.2)°	0.37
Lumipulse™/ Ella™	−6.3 (−8.8 to −3.1)	0.8 (0.7 to 0.9)	0.56

* The Cusum test for linearity confirms the applicability of the Passing-Bablok method if $p>0.05$.
° the hypothesis of similarity of test is accepted.

femtomolar concentrations (Rissin et al., 2010). Two other commercial platforms, Ella™ (Bio-Techne) and Lumipulse™ (Fujirebio), are becoming available for NfL. Ella™ is a microfluidic cartridge-based immunoassay platform measuring up to 72 samples in triplicate inside glass nanoreactors using a fluorescent substrate (Truffi et al., 2022). Lumipulse™ is an automated system based on a two-step sandwich chemiluminescent enzyme immunoassay (CLEIA) technology with medium/high throughput sample handling (Fujirebio Neuro Center of Excellence, 21AD). Few authors showed two-by-two differences between platforms (Gauthier et al., 2021; Truffi et al., 2022; Nötzel et al., 2022; Kuhle et al., 2016), and, to date, no performance comparisons between the three assays exist in the literature. Our study aimed to compare CSF and serum NfL levels determined with SIMOA™, Ella™, and Lumipulse™ in MS patients at diagnosis in order to define the degree of correlation between the results and evaluate the reliability of the new methods, Ella™ and Lumipulse™.

2. Methods

Sixty newly diagnosed relapsing-remitting MS patients (38 females; median age: 36.5 years, range: 15–60) were subsequently recruited at the University of Piemonte Orientale "Maggiore della Carità" University Hospital. They were enrolled at the time of their diagnostic work-up, and before steroid or disease-modifying treatment initiation. All patients signed study informed consents (local Ethics Committee approvals, Comitato Etico Interaziendale AOU "Maggiore della Carità" di Novara, ASL BI, ASL NO, ASL VCO: CE 060/2022 and 260/2022).

CSF and serum NfL values were determined with three platforms: the commercial Ella™ microfluidic platform (Bio-Techne), the Lumipulse™ fully automated system for the Chemiluminescent Enzyme Immuno-Assay (Fujirebio), and the commercial SIMOA™ on the SR-X instrument using NF-light assays (Quanterix). The Ella™ device was employed at "Maggiore della Carità" Hospital in Novara using Human NF-L Simple Plex assay (ProteinSimple, CA, USA) according to the manufacturer's instructions. The Lumipulse™ fully automated instrument was used at "Maggiore della Carità" Hospital in Novara using the Lumipulse G NfL CSF and Lumipulse G NfL Blood Chemiluminescent Enzyme Immuno-Assays. SIMOA™-based analysis was performed using NF-light assays (Quanterix) on the SR-X instrument at the Clinical Neurobiology Laboratory of the Multiple Sclerosis Regional Referral centre (CRESM, University Hospital San Luigi Gonzaga) at the Neuroscience Institute Cavalieri Ottolenghi, in Orbassano, and considered as the gold standard (Valentino et al., 2021; Revendova et al., 2022). Supplementary Table 1 summarizes the main features of the three techniques. All CSF and blood

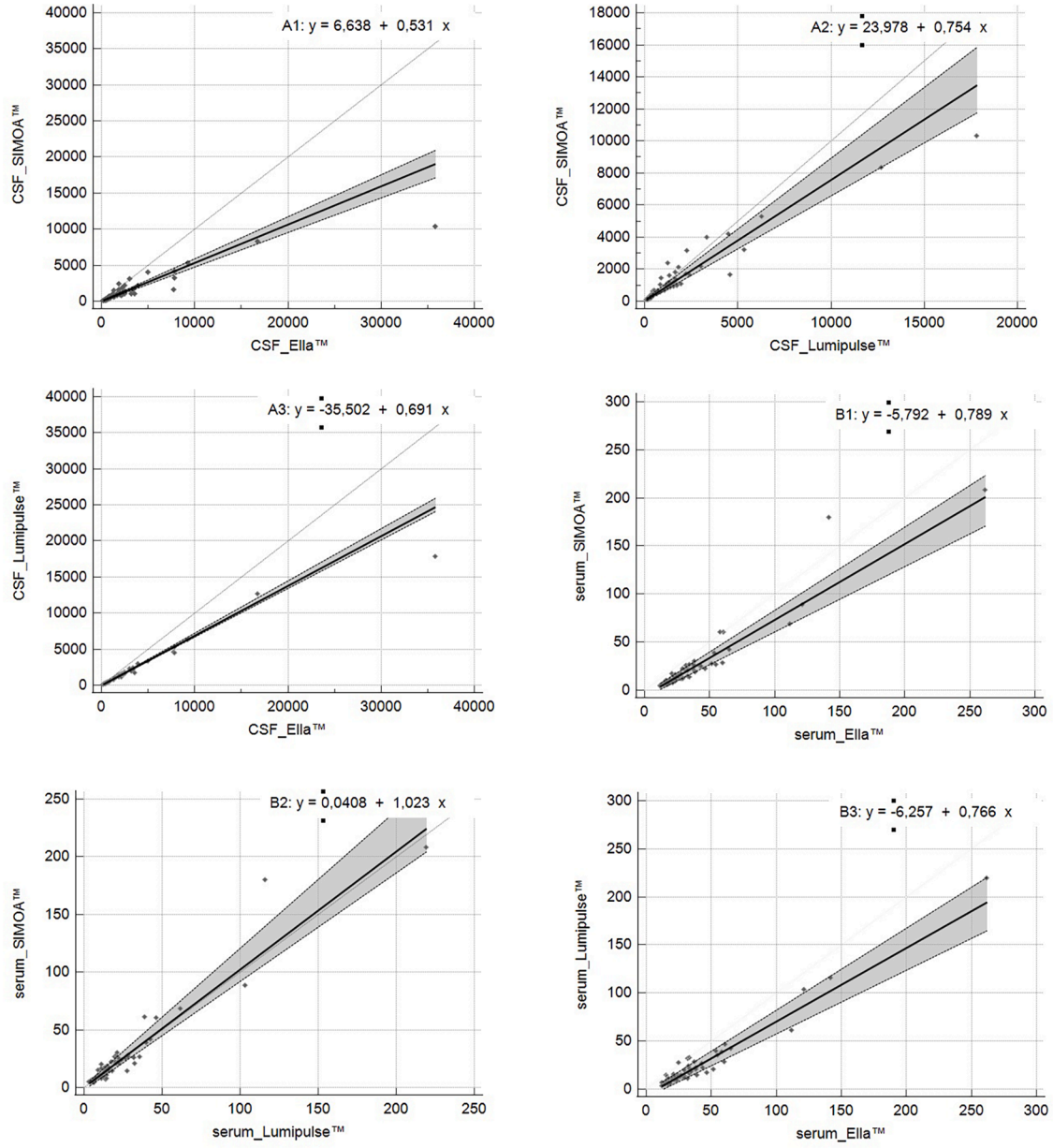

Fig. 2. Passing–Bablok regression analysis of CSF (A1–3) and serum (B1–3) NfL values measured by SIMOA™, Ella™, and Lumipulse™ in naïve MS patients. The lines represent the 95% limits of agreement.

samples were centrifuged upon arrival, and the supernatant was stored in aliquots at −80 °C. Before performing the assays, all samples were slowly thawed to 2–8 °C and then brought to room temperature. Statistical analysis was performed using SPSS 25.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc 22.009. Data were not normally distributed according to the Kolmogorov-Smirnov test and, consequently, non-parametric tests were employed. The Spearman's rank correlation coefficient test was used for the correlation between continuous variables. The Passing-Bablok regression and Bland–Altman methods evaluated agreement between assays. All tests were two-sided and the significance threshold was set at $p < 0.05$.

3. Results

Median CSF and serum NfL levels were, respectively, 1590.5 and 29.3 pg/ml with Ella™, 1105.0 and 14.7 pg/ml with Lumipulse™, 861.6 and 16.3 pg/ml with SIMOA™ (Fig. 1 and Table 1). As shown, the median NfL levels were higher in both serum and CSF if measured with Ella™ compared to Lumipulse™ and SIMOA™. However, values in both biological fluids strongly correlated between the two assays and SIMOA™ (Table 2: in all comparisons r >0.9; p <0.0001). NfL concentrations also correlated if measured with Ella™ and Lumipulse™, more strongly in the CSF (Spearman correlation: 0.98 (0.97–0.99); $p<0.001$) then in the serum (0.89 (0.89–0.94); $p<0.001$).

Afterwards, we compared the three assays with Passing-Bablok regression (Table 3). The analysis for CSF NfL evidenced a not-significant intercept and a minimal but significant slope considering both Ella™ and Lumipulse™ compared to SIMOA™: there was no constant but a proportional error between the two methods, which resulted higher for Ella™. Regarding serum Nfl, there was neither constant nor proportional difference only between SIMOA™ and Lumipulse™, thus confirming a very high agreement between methods. The scatter diagram and regression lines are in Fig. 2. The Bland-Altman comparison of multiple methods confirmed the agreement between the two assays and SIMOA™, used as the reference method (Table 4). The analysis also showed lower biases for CSF and serum NfL detected with Lumipulse™

Table 4
Bland-Altman plots for SIMOA™ (as reference method), Ella™, and Lumipulse™.

Comparison between methods	Bland-Altman for multiple methods (SIMOA™ as reference method)			
	Bias (pg/ml) (95% CI)	Lower limit (pg/ml) (95% CI)	Upper limit (pg/ml) (95% CI)	% difference (95% CI)
CSF				
Ella™ - SIMOA™	1417.9 (511.4 to 2324.4)	−5459.9 (−7017.8 to −3902.1)	8295.7 (6737.8 to 9853.5)	58.6 (50.7 to 66.4)
Lumipulse™ - SIMOA™	405.7 (89.3 to 722.0)	−1994 (−2538.2 to −1450.9)	2805.9 (2262.2 to 3349.5)	18.2 (10.5 to 25.9)
Ella™ - Lumipulse™	1012.2 (397.0 to 1627.5)	−3655.8 (−4713.1 to −2598.4)	5680.2 (4622.9 to 6737.5)	42.5 (39.9 to 45.2)
Serum				
Ella™ - SIMOA™	12.9 (9.9 to 15.9)	−9.6 (−14.6 to −4.5)	35.4 (30.3 to 40.5)	55.5 (48.7 to 62.4)
Lumipulse™ - SIMOA™	−1.5 (−4.1 to 1.2)	−21.3 (−25.8 to −16.8)	18.4 (13.9 to 22.9)	−3.2 (−9.9 to 3.4)
Ella™ - Lumipulse™	14.4 (12.0 to 16.7)	−3.6 (−7.7 to 0.4)	32.4 (28.3 to 36.4)	58.2 (50.8 to 65.5)

(than Ella™) if compared to SIMOA™. Plots are shown in Fig. 3.

4. Discussion

In our cohort of MS naïve patients, we compared CSF and serum NfL levels measured by SIMOA™, Lumipulse™, and Ella™. We confirmed that CSF values exceed those in the serum, resulting from 52- to 75-fold higher using, respectively, Lumipulse™ or SIMOA™[9,19,20]. Globally, serum and CSF concentrations detected with Lumipulse™ and Ella™ strongly correlated with the SIMOA™ ones, used as the gold standard. Regarding CSF, Lumipulse™ and Ella™ overestimated the levels measured with SIMOA™, being the Lumipulse™ results (bias +405.7 pg/ml) more similar to the gold standard than those with Ella™ (bias +1417.9 pg/ml). Serum NfL values measured with Ella™ were also significantly higher than those obtained with SIMOA™ (bias +12.9 pg/ml), whereas the levels measured with Lumipulse™ overlapped more precisely (bias −1.5 pg/ml) with the gold standard. The differences tend to be more evident at high NfL concentrations, both in CSF and serum, as already reported (Nötzel et al., 2022). Gauthier et al. (2021) suggested that the differences between Ella™ and SIMOA™ could be ascribed to the different calibrators, which are naturally derived bovine NfL for Ella™ and recombinant human NfL for SIMOA™, but it must be noted that also Lumipulse™ uses naturally derived bovine NfL. Our study is limited to the MS population, and we did not perform repeated measurements during the disease course limiting our selection to naïve MS cases before any treatments that could modify NfL values (Piehl et al., 2018; Novakova et al., 2017; Delcoigne et al., 2020).

So far, no other comparisons have been reported previously between the three assays to quantify NfL levels in serum and CSF, since comparisons were available between Ella™ and SIMOA™, but not using Lumipulse™. Previous works showed that Ella™ overestimated serum NfL if compared to SIMOA™ in 42 MS patients treated with alemtuzumab (Nötzel et al., 2022) and in 203 French patients in different phases of disease and treatments (Gauthier et al., 2021), while it underestimated serum NfL in a cohort of 32 cases (Revendova et al., 2022). Nonetheless, Truffi et al. also showed a strong correlation between SIMOA™ and Ella™ in determining NfL plasmatic values in patients with dementia, being the concentrations 17% higher using Ella™ (Truffi et al., 2022). We did not find any previous report on Lumipulse™, whereas some data are available for Ella™ compared to SIMOA™. Most studies used the SIMOA™ ultrasensitive method (Disanto et al., 2017; Ferreira-Atuesta et al., 2021; Piehl et al., 2018; Khalil et al., 2020; Novakova et al., 2018), and reference values are limited to this assay (Valentino et al., 2021). Consequently, clinicians must pay attention when using data and defining them as pathological if the analysis was performed with the other methods. Each platform has its own advantages and disadvantages. SIMOA™ boasts the lowest limit of quantification (LOQ), 0.174 pg/mL, which makes it particularly suitable in patients who are expected to have very low NfL concentrations (Nötzel et al., 2022). Compared to Ella™, whose cartridges are single-use only, SIMOA™ assays are more flexible in use. However, SIMOA™ costs often limit its widespread availability in clinical laboratories, whereas the ELLA™ instrument is cheaper and more easily portable (Revendova et al., 2022; Gauthier et al., 2021; Nötzel et al., 2022). The Lumipulse™ instrument has the great advantage of providing a fully automatic assay with minimal preanalytical procedures, and its costs are between those of SIMOA™ and ELLA™.

In conclusion, NfL are becoming an established biomarker to monitor MS activity over time (Nötzel et al., 2022; Siller et al., 2019). Baseline values at diagnosis are needed to be compared with subsequent measures to monitor the disease course. Although CSF values resulted higher than the serum ones with all the assays, their usefulness is limited for monitoring MS patients since repeated lumbar puncture procedures would be required over time. By contrast, serum NfL measurements are acceptable for the patient, and could expand the use of this assay in clinical routine. All available techniques are effective in detecting serum NfL, even though some differences in results must be considered in clinical practice when comparing different trials. In our cohort, we evidenced the best agreement between SIMOA™ and Lumipulse™, especially for serum values.

CRediT authorship contribution statement

D Vecchio: Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Software, Validation, Writing – original draft. **C Puricelli:** Data curation, Formal analysis, Methodology, Software, Visualization, Writing – original draft. **S Malucchi:** Data curation, Methodology, Supervision, Validation, Writing – review & editing. **E Virgilio:** Conceptualization, Data curation, Methodology, Writing – review & editing. **S Martire:** Data curation, Formal analysis, Investigation, Software, Writing – review & editing. **S Perga:** Data curation, Methodology, Project administration, Resources, Writing – review & editing. **F Passarelli:** Formal analysis, Investigation, Software. **P Valentino:** Data curation, Formal analysis, Methodology, Resources, Writing – review & editing. **A Di Sapio:** Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing. **R Cantello:** Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing. **U Dianzani:** Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **C Comi:** Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

Conflicts of interest/Competing interests: none for all authors for this work; Ethics approval: local Ethical Committee approvals (Comitato Etico Interaziendale AOU "Maggiore della Carità" di Novara, ASL BI, ASL

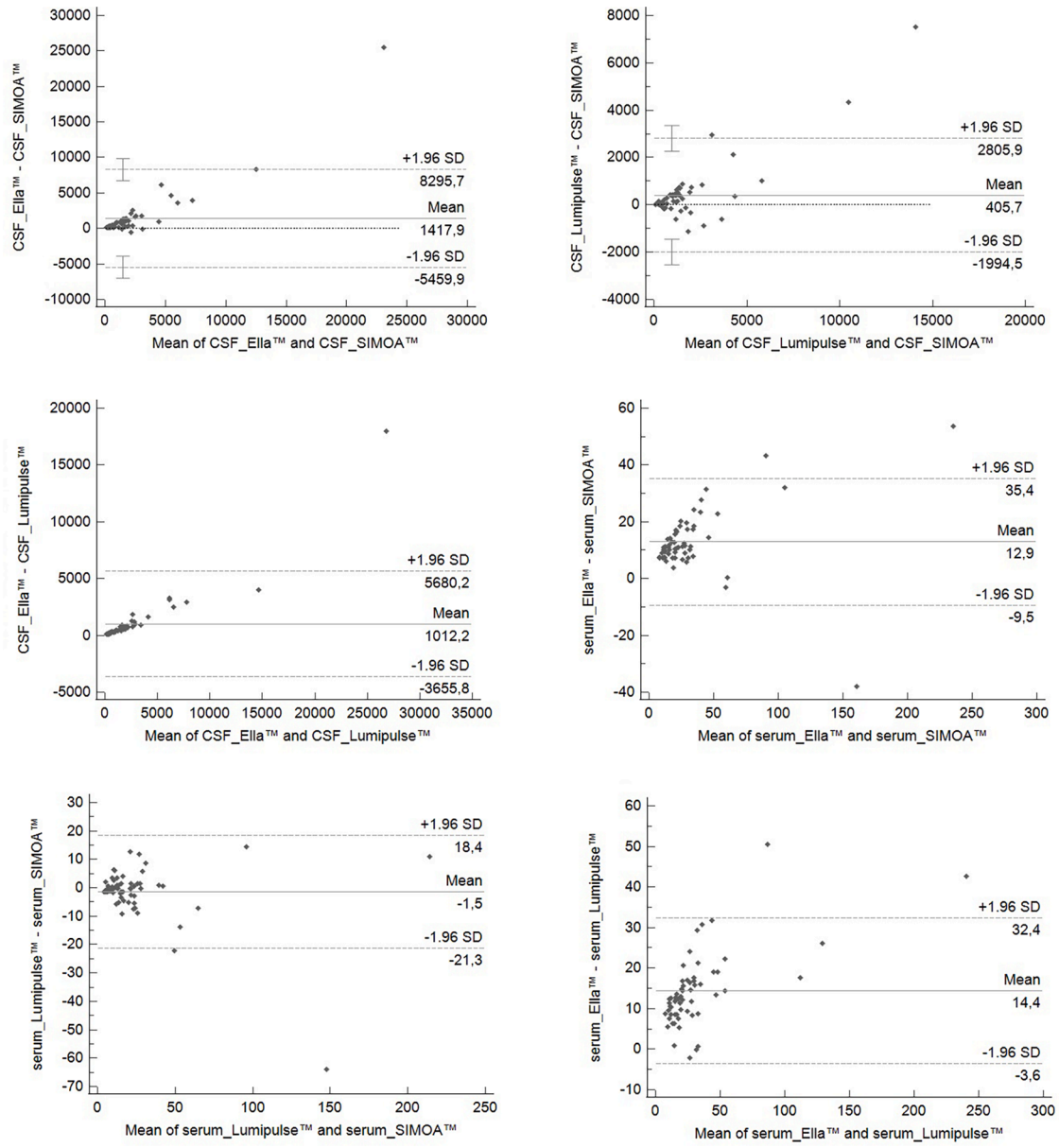

Fig. 3. Bland-Altman plots with differences between the two methods against the averages of the two methods. The black dotted line represents the line of equality.

NO, ASL VCO): CE 060/2022 and 260/2022). Consent to participate/consent for publication: written consent obtained from all participants; Availability of data and material: data available on request.

Acknowledgments/Funding

Fujirebio partially supported for the use of their materials. Roche supported SIMOA™ material acquisition in the "Rete Nord-Ovest" project for NfL dosage.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.msard.2023.105412.

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