Neurofilament Light Chain Measurements by Centaur and Simoa Systems in Human and Murine Samples

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ABSTRACT **Background:** Neurofilament light chain (NfL) is an established biomarker for detecting axonal injury in various neurological disorders. The Quanterix Single Molecule Array (Simoa) is the current standard; however, automated immunoassays, such as the Siemens Atellica and Centaur, may serve as alternatives.

Objectives: To compare NfL measurements obtained with the Centaur system to those from the Simoa-SR-X. To assess their agreement and applicability in clinical practice, research, and animal studies.

Methods: NfL levels were measured in 27 human serum, 8 plasma and 16 cerebrospinal fluid (CSF) samples, and 9 murine serum samples, by Centaur and Simoa systems. NfL levels in concomitantly drawn serum and plasma were compared in 8 humans. The agreement between platforms was evaluated.

Results: NfL levels measured by Centaur and Simoa systems demonstrated a strong correlation in serum (Spearman r=0.97, $P < 0.0001$) and plasma (Pearson $R^2=0.95$, $P < 0.0001$). Centaur measurements were higher ($P = 0.01$) than Simoa. Most importantly, system-specific Z-scores corrected these differences. Serum and plasma levels measured by the Centaur system correlated strongly ($R^2=0.98$, $P < 0.0001$) and showed similar results. CSF levels measured by the Centaur system were lower (52% bias) than those measured by Simoa, with poor correlation at concentrations within the normal range ($R^2=0.32$, $P = 0.11$). Mouse serum results showed a strong correlation between systems ($R^2=0.86$, $P < 0.001$) with similar values.

Conclusions: The Centaur system offers an alternative to Simoa for measuring NfL in human serum, plasma, and murine serum. System-specific age-adjusted Z-scores are essential for interpretation. CSF evaluation requires further assessment.

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Axonal injury is a key process in many neurological disorders, and there is a growing clinical need for accessible, reliable biomarkers for its detection and monitoring. Neurofilament light chain (NfL) has emerged as one of the most promising markers, with proven value in diagnosis, prognosis, disease monitoring, and treatment assessment across conditions such as multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis, stroke, peripheral neuropathies, and traumatic brain injury [1].

The Ultra-sensitive Quanterix Single Molecule Array (Simoa) system (Quanterix Corporation, USA) [2] has transformed NfL testing, enabling measurement in serum and plasma. It has also supported the development of age-adjusted reference values [3]. However, Simoa is costly, technically demanding, and available only in a small number of specialized centers in Israel and worldwide, which limits its integration into routine clinical care. The ADVIA Centaur® XPT Immunoassay System (Siemens Healthineers, Erlangen, Germany), which is already in use in many hospital laboratories worldwide and across Israel, offers a potential solution, enabling rapid, automated, and cost-effective NfL testing. Previous studies compared Simoa with newer automated systems [4-6]. However, validating Centaur against Simoa could significantly expand national access to this biomarker and improve patient care.

In this study, we compared NfL measurements by the Centaur and Simoa systems in human serum, plasma, and cerebrospinal fluid (CSF), as well as murine serum, to evaluate their agreement and the feasibility of broader clinical and research implementation.

PATIENTS AND METHODS

PATIENTS AND CONTROLS

Blood and CSF samples were collected from patients and volunteers following written informed consent (SMC 6526-19; SMC 7786-20). This study was approved by the Sheba Medical Center Institutional Review Board. Blood samples were incubated at room temperature (RT) for 30–60 minutes and centrifuged at 1500 × g for 10 minutes. Serum or plasma was removed and stored at -80°C. CSF was stored under the same conditions.

MICE

Adult male ICR mice blood was collected immediately following euthanasia and decapitation according to institutional and national guidelines, approved by the Sheba Animal Care and Use Committee (protocol #1296-21-ANIM).

SERUM, PLASMA AND CSF NFL MEASUREMENTS

Simoa assays

Levels were measured in duplicates using the NF-light Advantage kit V1, V2, and V2 Advantage Kit Plus. Human serum and plasma samples were manually diluted at a 1:4 ratio, as recommended by the manufacturer. This procedure allowed a reportable range of 0.345–360 pg/ml. Additional dilutions were manually performed if needed. CSF samples were diluted 1:100 using the same procedure. Mouse serum samples were diluted 1:100. Age-adjusted Z-scores were calculated using a previously detailed Simoa-derived formula (Simoa-specific Z-scores) [7].

Centaur assays

Samples were analyzed using the NfL OUS-ADVIA assay kit. Undiluted human serum, plasma, and CSF samples (160 µL) were loaded. This procedure allowed a reportable range of 2.5–300 pg/ml. Additional dilutions were performed automatically by the system if needed. Age-adjusted Z-scores were calculated by using the R Shiny web application [8], derived from measurements obtained by the Siemens Atellica system (Atelica/Centaur-specific Z-scores).

STATISTICAL ANALYSIS

The normality of each dataset was evaluated using the Anderson-Darling test. Pearson and Spearman correlation tests and linear regression analysis were performed. Independent and paired two-tailed *t*-tests were applied to the normally distributed datasets. The Mann-Whitney or Wilcoxon tests were applied to the non-normally distributed datasets. The results are expressed as the median, interquartile range (IQR), and the mean ± standard error of mean (SEM). *P*-values < 0.05 were considered significant. The statistical analysis was performed with GraphPad Prism version 8 (GraphPad Software, USA).

RESULTS

HUMAN SERUM

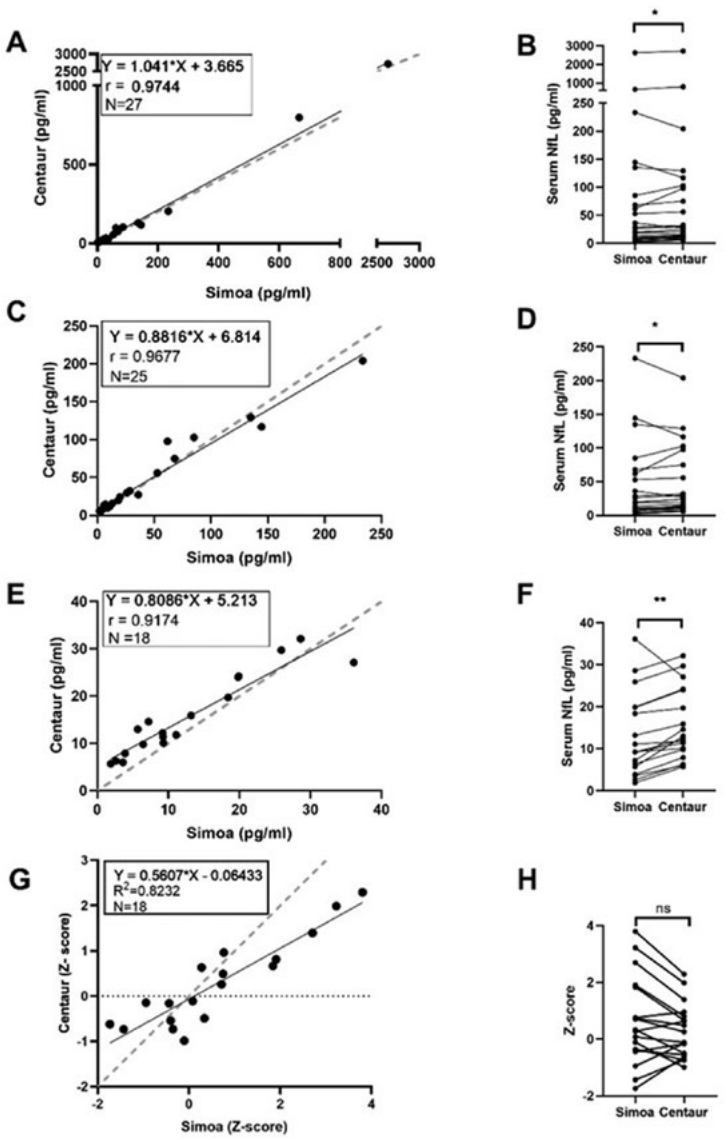
A total of 51 human blood and CSF samples (ages 18–82, 57% male) were analyzed using both the Simoa and Centaur systems. Samples were derived from 32 patients with various neurological diseases, including immune-mediated, degenerative, and amyloid-related disorders. We also included 13 healthy participants without a diagnosis. Serum and plasma from 8 cases were available for comparison by the Centaur system.

Centaur and Simoa NfL measurements showed a strong and significant correlation (r=0.97, $P < 0.0001$, n=27, Spearman Correlation test) across the entire NfL level range tested [Figure 1A]. However, the median and the interquartile range (IQR) of NfL levels measured by the Centaur system were higher than those measured with the Simoa system (median [IQR] 24 [11–97.9] vs. 19.8 [7.2–68] pg/ml, respectively, Wilcoxon *t* test, $P = 0.01$) [Figure 1B].

A repeated analysis of samples with results at the spectrum below the upper measurement limit of the undiluted samples utilizing the Centaur system (< 300 pg/ml) showed a strong and significant correlation (r=0.98, $P < 0.0001$, n=25) [Figure 1C]. Within this range, the pattern remained similar, with Centaur measurements higher than those from the Simoa (median [IQR] 19.70 [10.7–65.4] vs. 18.41 [6.8–57.3] pg/ml, respectively, Wilcoxon *t*-test, $P = 0.036$) [Figure 1D]. We then repeated this analysis with samples that showed NfL levels below 40 pg/ml, which is the most clinically relevant range for differentiating between normal and abnormal results. At this range, results showed a strong and significant correlation (r=0.92, $P < 0.0001$, n=18) [Figure 1E]. A similar trend of higher values measured by the Centaur system compared to the Simoa was observed in this range (median [IQR] 12.60 [9.3–24.0] vs. 9.270 [5.2–19.8] pg/ml, respectively, Wilcoxon *t*-test, $P = 0.002$) [Figure 1F]. To determine the clinical relevance of the differences in results, system-specific Z-scores were calculated for the measured values. Simoa and Centaur Z-scores showed a significant correlation

Figure 1. Correlation and comparison of NfL measurements in human serum using the Sioma and Centaur systems across a spectrum of 0–3000 pg/ml **[A,B]**, 0–250 pg/ml **[C,D]**, 0–40 pg/ml **[E,F]**; and for system-specific calculated z-scores **[G,H]**. For illustration, a linear dense regression line and a dashed identity line are shown

NfL = neurofilament light chain
*P < 0.05
**P < 0.01



(Pearson R^2=0.82, P < 0.0001, n=18) [Figure 1G], with no significant difference between the mean Z-scores of the two systems (mean ± SEM: 0.2783 ± 0.2254 vs. 0.6112 ± 0.3648, respectively; paired *t*-test, P = 0.09) [Figure 1H].

HUMAN PLASMA SAMPLE MEASUREMENTS

NfL levels in plasma were compared between the Simoa and Centaur systems in eight cases and showed a strong and significant correlation (R^2=0.95, P < 0.0001) [Figure 2A]. Similar to serum measurements, the median and the IQR of NfL levels measured by the Centaur were higher than those measured with the Simoa system (median [IQR] 23.85 [11.48–34.88] vs. 15.13 [8.07–26.67] pg/ml, respectively, paired two-tailed *t*-test, P = 0.0018).

To further determine whether serum and plasma samples could be used interchangeably by the Centaur system, we compared NfL measurements of plasma and serum of these eight cases, which were collected at the same blood draw. This comparison revealed a strong and significant correlation between measurements (R^2= 0.98, P < 0.0001) [Figure 2B]. The median and the IQR of NfL levels of serum and plasma measurements were similar (median

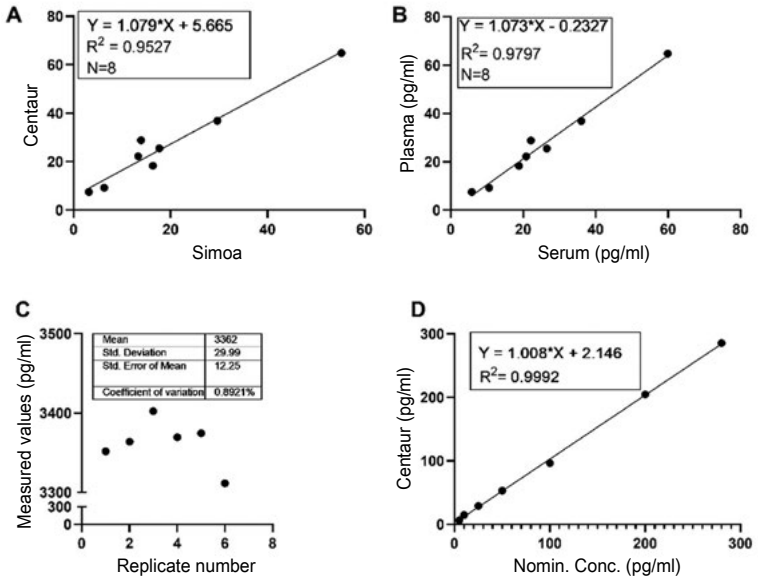


Figure 2. Comparison and validation of NfL measurements in human serum and plasma.

[A] Correlation of plasma samples tested by the Centaur and Simoa systems. **[B]** Correlation of serum and plasma samples measured by the Centaur system. For illustration, a linear regression line is shown [in A and B]. **[C]** Reproducibility of high NfL level measurements in human serum tested by repeated measurements of the same sample and **[D]** linearity assessment by serial dilutions.

NfL = neurofilament light chain

[IQR] 21.45 [12.65–33.63] vs. 23.85 [11.48–34.88] pg/ml, respectively, paired two-tailed *t*-test, $P = 0.161$).

REPEATABILITY AND PRECISION OF THE CENTAUR SYSTEM

To assess the repeatability and precision of the Centaur system at high NfL levels, we performed six repeated measurements of a representative human plasma sample. The calculated coefficient of variation (CV%) was 0.89%, demonstrating good reproducibility under the test conditions [Figure 2C].

To evaluate linearity, a representative serum sample was serially diluted across six concentrations, and NfL levels were measured. The measured concentrations showed a strong linear correlation with the expected nominal values (R^2=0.99, $P < 0.001$), as indicated by the regression equation Y=1.008*X+2.146, demonstrating linearity across the tested range [Figure 2D].

HUMAN CSF SAMPLES

CSF has a distinct fluid composition compared to both serum and plasma; therefore, its assay applicability requires validation. Furthermore, the level of NfL in CSF is approximately 40–60 times higher than that in blood [9] and therefore may require dilution before assay performance.

Prior to testing CSF in the Simoa system, the sample was manually diluted 1:100, according to the manufacturer's instructions. The Centaur system performs dilution automatically when NfL levels are above 300 pg/ml. We measured the NfL levels of 16 human CSF samples using both the Simoa and Centaur systems. Measurements showed a strong and significant correlation between the two systems (r=0.84, $P < 0.0001$) [Figure 3A]. However, a significant bias was detected, with results measured by the Centaur being 52% lower than the Simoa assay results (slope of 0.48, 95%CI 0.44–0.52). Re-analysis of NfL levels at a range below 500 pg/ml, which relates to normal levels, showed a reduced, moderate, and non-significant correlation (R^2=0.32, $P = 0.11$) [Figure 3B]. A CSF Z-score comparison was not performed as such data is not available for the Centaur system.

MOUSE SERUM SAMPLES

In previous studies, we demonstrated that measuring serum NfL levels in mice is an effective research marker of neuronal loss [10,11], similar to its use in humans. To compare the performance of the two systems for animal studies, we measured NfL in nine mouse serum samples using both the Simoa and Centaur systems. A strong and significant correlation was observed between the two systems (R^2=0.86, $P < 0.001$) [Figure 3C]. The median and the IQR of NfL levels measured by the Centaur system were similar to those of with the Simoa system (median [IQR] 168.8 [78.65–232] vs. 175 [90.39–266.6] pg/ml, respectively, paired two-tailed *t*-test, $P = 0.31$).

DISCUSSION

In this study, we compared NfL levels measured by the Siemens Centaur system and the Quanterix Simoa system. We found strong correlations between systems for human serum and plasma, although Centaur consistently reported higher values. Applying age-adjusted Z-scores using system-specific normative data corrected these differences. Our results indicate that Z-score calculation is required to compare patient results between the two systems, which allows implementation of the Centaur system as a cost-effective and accessible alternative for routine clinical use. Serum and plasma results were comparable on Centaur, and murine serum measurements showed good agreement with Simoa, supporting its use in translational research.

Although both systems use the same primary monoclonal antibodies [5,12], differences in calibrators, reagents,

dilution protocols, and hardware contribute to systematic variation. Standardization through system-specific Z-scores corrects for these differences, but careful interpretation is essential, particularly when results come from different systems, kit lots, or laboratories. Centaur's calibration is not batch-dependent, offering greater consistency across runs.

CSF measurements showed greater variability between platforms, likely due to manual dilution steps required for Simoa versus full automation on Centaur, as well as possible effects from the differing sample compositions. The absence of a certified reference for CSF NfL further limits standardization, and larger studies are needed to establish validated reference values and age-adjusted models for CSF.

The Centaur system offers key advantages, including random-access operation, wide availability in Israeli hospitals and globally, lower cost, and CE-mark regulatory approval, making it well-suited for clinical integration and repeated patient monitoring. These benefits extend beyond neurology to other specialties where NfL may aid in diagnosis and prognosis.

Limitations of our study include the modest sample size, especially for plasma and CSF. Murine analysis was limited to serum. Further validation in additional sample types and species is warranted. We used the Simoa SR-X rather than the higher-throughput, fully automated HD-X, reflecting the cost constraints common to most clinical laboratories.

CONCLUSIONS

The Siemens Centaur NfL assay provides clinically comparable results to Simoa for serum and plasma, with added advantages of automation, affordability, and accessibility. Its applicability to both human and murine samples supports broader adoption in clinical neurology and translational research. Future work should focus on refining cross-platform CSF reference values and expanding validation in diverse animal model settings.

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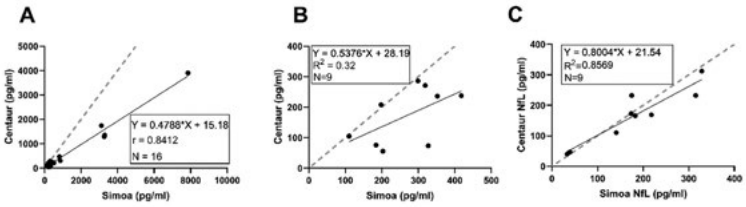
Figure 3. Comparison of NfL levels in human CSF and mouse serum measured using the Simoa and Centaur systems. Correlation comparison **[A]** across the entire concentration range, below 500 pg/ml in **[B]** human CSF and **[C]** mouse serum. For illustration, a linear regression line is shown. The dashed line indicates the line of identity.

NfL = neurofilament light chain



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