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Method comparison of plasma and CSF GFAP immunoassays across multiple platforms

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Abstract

Objectives: Glial fibrillary acidic protein (GFAP) is a biomarker of astrocytic activation associated with neurodegenerative diseases, neuroinflammatory disorders, and traumatic brain injury. However, the lack of standardized methods for quantifying GFAP across different immunoassay platforms poses challenges for its clinical implementation. This study aimed to compare the analytical performance of multiple commercially available and in-house immunoassays for GFAP quantification in plasma and cerebrospinal fluid (CSF) to assess their agreement and potential interchangeability.

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Methods: We conducted a method comparison using four plasma GFAP immunoassays (Simoa, Ella, Alinity, and MSD) and four CSF GFAP assays (ELISA, Ella, Alinity and MSD). Anonymized leftover plasma and CSF samples were analyzed across platforms. Sample sizes for the pairwise comparisons ranged from 23 to 52 for plasma and 34 to 51 for CSF. Pairwise comparisons were performed using Spearman correlation, Bland–Altman analysis, and Passing–Bablok regression to assess systematic and proportional biases. Outliers were identified and excluded to ensure robust statistical evaluation.

Results: Strong correlations were observed across all platforms (Spearman's $r=0.827$ – 0.927 for plasma; $r=0.937$ – 0.958 for CSF). However, significant systematic and proportional biases were present in several comparisons, preventing direct interchangeability of results. In plasma, Simoa consistently reported higher GFAP concentrations compared with Ella and Alinity, while Alinity overestimated levels relative to Ella. Similarly, in CSF, ELISA tended to underestimate GFAP concentrations compared with Alinity, MSD, and Ella, with the largest discrepancy observed between ELISA and MSD.

Conclusions: Despite strong correlations, substantial method-dependent biases indicate that GFAP measurements across different immunoassay platforms need to be standardized to ensure harmonization and reliable clinical application of GFAP as a biomarker.

Keywords: glial fibrillary acidic protein (GFAP); biomarker; immunoassay; plasma; cerebrospinal fluid; method comparison

Introduction

Glial fibrillary acidic protein (GFAP) is a type-III intermediate filament protein predominantly expressed by astrocytes in the central nervous system (CNS) [1]. Beyond its physiological roles, such as providing structural support to astrocytes and contributing to the maintenance of the blood-brain barrier, GFAP expression is upregulated in response to neuronal injury, serving as a marker of astrocytic activation following CNS damage [2]. Due to the close proximity of CSF to the brain parenchyma, GFAP concentrations in CSF are significantly higher than in blood, with levels approximately 100 times

greater in CSF. Consequently, fluctuations in GFAP levels might be more readily detected in CSF than in blood, although large changes can also be observed in plasma despite its lower absolute concentrations [3, 4]. Moreover, the correlation between plasma and CSF GFAP levels has been reported to be only moderate [5, 6]. This has been partly attributed to the limited long-term stability of GFAP in CSF [7], particularly in scenarios involving multiple freeze–thaw cycles. Another proposed explanation is the hypothesis that GFAP released through astrocytic end-feet in direct contact with the vascular structures may contribute more directly to blood GFAP levels, bypassing the CSF compartment altogether [5, 8].

Given the concentration differences between CSF and plasma, measuring GFAP in blood was historically challenging. However, recent advancements in detection technologies over the past decade have enabled the measurement of low-abundance, brain-derived proteins in blood that were previously undetectable using conventional enzyme-linked immunosorbent assays (ELISA) [9, 10]. These innovations have facilitated the detection of low abundant proteins in blood, such as GFAP, using highly sensitive immunoassay platforms, including single-molecule array (Simoa) [11], Ella Simple Plex [12], Meso Scale Discovery (MSD) (e.g., MESO QuickPlex® SQ) [13]. Furthermore, as laboratories worldwide strive to implement blood GFAP assays in routine clinical practice, the need for high-throughput, fully automated immunoassays that can be seamlessly integrated into clinical chemistry laboratories has become increasingly important. In line with this, Abbott has developed a GFAP assay compatible with the Alinity platform [14], offering a potential solution for large-scale clinical chemistry laboratories, and many more assays, including those from Fujirebio [15], are on the way. These assays have the potential to aid clinicians in biomarker-supported diagnostic algorithms and assist in the differential diagnosis of complex neurological disorders.

With the availability of sensitive assays, many studies have demonstrated elevated GFAP levels in both CSF and blood across a variety of neurological conditions, including neurodegenerative diseases (e.g., Alzheimer's disease (AD) [5]), neuroinflammatory disorders (e.g., multiple sclerosis [16]), and traumatic brain injury (TBI) [17]. Overall, GFAP serves as a valuable biomarker for supporting clinical diagnosis across a broad spectrum of neurological disease contexts.

Given its growing clinical relevance and the increasing availability of assays for quantifying GFAP in biological fluids, the need for standardization and comparative data to evaluate immunoassay platforms and assess their agreement has become increasingly important. While Simoa is widely utilized and integrated into routine laboratory practice, the assessment and adoption of alternative methods and platforms are crucial for ensuring result

harmonization. Although commercially available immunoassay instruments are based on the fundamental principles of sandwich immunoassays, the absence of certified reference materials, variations in antibody pair selection, assay design, the use of different calibrators, and instrument-specific characteristics pose challenges for standardizing result interpretation across laboratories using different methodologies. To address these limitations, this study presents a method comparison across multiple currently available immunoassay platforms for both plasma (Simoa, Ella, Alinity, MSD) and CSF (ELISA, Ella, Alinity, MSD), providing a comprehensive evaluation of their agreement.

Materials and methods

Study design

Anonymized leftover plasma and CSF samples, originally obtained for routine analyses at the Neurochemistry and Clinical Chemistry Laboratories at Sahlgrenska University Hospital, were used for method comparison experiments. Due to differences in assay availability and sample volume constraints at the time of analysis, a unified pooled sample set could not be used across all pairwise comparisons. Instead, we collected separate sets of leftover samples for each comparison. These sets were selected to cover a comparable range and distribution of GFAP concentrations. The variation in sample numbers reflects practical limitations, including assay scheduling requirements and differences in leftover sample availability over time. Before conducting the method comparison analyses, we visually assessed the range and distribution of GFAP concentrations for the same assays used in multiple pairwise comparisons (plasma and CSF) to provide an overview of absolute GFAP concentrations across datasets prior to outlier exclusion (Supplementary Figures 1–3).

Ethics approval and consent to participate

The collection of anonymized samples at the Clinical Chemistry Laboratory, Sahlgrenska University Hospital, was carried out in compliance with the ethical guidelines approved by the Ethics Committee at the University of Gothenburg (EPN140811).

Study participants and sample collection

In this study, only anonymized leftover and randomly collected samples were used, with no access to clinical or

demographic data. The detailed number of samples used in each specific experiment can be found in the corresponding section of this manuscript. To separate plasma from whole blood, samples underwent an initial centrifugation at $2,000\times g$ for 10 min. Prior to analysis, an additional centrifugation step was performed for Ella, MSD, and Simoa assays, while the centrifugation protocol for Alinity was verified against the kit insert. CSF samples were collected in polypropylene tubes, centrifuged at $1,800\times g$ for 10 min at 14°C , and the supernatant was stored at -20°C for a maximum of one week until biomarker analysis. At the time of analysis, samples were vortexed before measurement.

Immunoassays and the number of samples included in each pairwise comparison

Different platforms were utilized to compare plasma and CSF GFAP levels using various numbers of paired samples for pairwise comparisons. For both matrices, the following assays were utilized: the Simple Plex Human GFAP (2nd Gen) Assay Cartridge on the Ella platform (Bio-Techne), the GFAP assay included in Abbott's mild traumatic brain injury (mTBI) panel alongside UCH-L1 on the Alinity platform (Abbott), and the S-PLEX Human GFAP Kit on the MESO QuickPlex SQ[®] platform (MSD). Additionally, plasma GFAP was measured using the Simoa[®] GFAP Discovery Kit on the HD-X platform (Quanterix), while CSF GFAP levels were also assessed using an in-house developed ELISA [18] on the Sunrise microplate reader (Tecan Trading AG). For plasma GFAP, method comparisons were performed across four immunoassays, with the following pairwise comparisons: Ella vs. Simoa ($n=104$), Alinity vs. Ella ($n=40$), Simoa vs. MSD ($n=35$), and Simoa vs. Alinity ($n=39$). For CSF GFAP, comparisons included ELISA vs. Alinity ($n=54$), ELISA vs. MSD ($n=36$), and ELISA vs. Ella ($n=53$). After excluding outliers, the statistical analysis included: Alinity vs. Ella ($n=38$), Simoa vs. Alinity ($n=39$), Simoa vs. MSD ($n=34$), and Ella vs. Simoa ($n=102$) for plasma GFAP, while for CSF GFAP, the comparisons were ELISA vs. Alinity ($n=48$), ELISA vs. MSD ($n=34$), and ELISA vs. Ella ($n=51$). Additionally, following the identification of results below the lower limit of quantification (LLOQ) for Ella and their potential impact on the pairwise comparisons, values below the LLOQ were excluded from the final statistical analysis. Consequently, the updated analysis included 23 samples for the Alinity vs. Ella comparison and 52 samples for the Ella vs. Simoa comparison.

The technical specifications of immunoassays used for GFAP quantification

For both plasma and CSF method comparison, we evaluated multiple immunoassay platforms to assess analytical performance and agreement. The immunoassays used in this study are summarized in Table 1, including key assay specifications such as assay technology, measurement range, lower limit of quantification (LLOQ), and lot numbers.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 9.5.1 (San Diego, USA) and MedCalc statistical software version 23.1.3 (MedCalc Software Ltd, Belgium). For each immunoassay platform, data distribution was assessed, and outliers were identified separately for plasma and CSF measurements, using Tukey's fences [19] – a method based on the interquartile range (IQR) – prior to statistical analysis. Following assessment of data normality using the D'Agostino–Pearson test, non-parametric tests were used for further analyses. Bland-Altman [20] plots (% difference vs. average, where % difference = $100 \times (\text{assay 1} - \text{assay 2}) / \text{average of the two assays}$), Passing–Bablok [21] regression analysis, and Spearman correlation were employed for method comparison. Fixed bias was defined to be present if the 95 % confidence interval (CI) of the intercept did not include 0, while proportional bias was defined to be present if the 95 % CI of the slope did not include 1 [22].

Results

Spearman correlation analysis across the entire cohort demonstrated strong, positive correlations between all plasma and CSF GFAP assays, while pairwise comparisons revealed the presence of proportional bias, fixed bias, or both (Table 2).

Method comparison of plasma assays

A strong correlation was observed between the Alinity and Ella assays ($r_s=0.839$, $p<0.0001$). Bland-Altman analysis indicated a mean difference of 113.8 % (95 % CI: 107.6–119.9 %), with limits of agreement ranging from 85.8 % (95 % CI: 75.1–96.5 %) to 141.7 % (95 % CI: 131.0–152.4 %). These results indicate that Alinity consistently reported higher GFAP

Table 1: The technical specifications of immunoassays used for GFAP quantification.

Assay platform	Sample type	Technology	Instrumentation	Measurement range, ng/L	LLoQ ^a , ng/L	Dilution factor	CV%	Lot number	Additional notes
Simoa	Plasma	Single molecule array	Quanterix HD-X	0–4,000	0.686	Plasma 4× CSF 40×	Low: %CV _r : 6.2 %CV _{Rw} : 6.2 High: %CV _r : 3.3 %CV _{Rw} : 3.3 Plasma Low: %CV _r : 14.3 %CV _{Rw} : N/A High: %CV _r : 2.5 %CV _{Rw} : N/A CSF Low: %CV _r : 1.3 %CV _{Rw} : 6.3 High: %CV _r : 4.8 %CV _{Rw} : 9.6 N/A ^b	REF 102336	E.g., Automated, plate-based platform, multiplexing capacity
Ella	Plasma/CSF	Microfluidic cartridge containing three glass nano reactors, fluorescence readout	Ella™ Automated ELISA	2.52–9,600	5.1	CSF 2×		41,689 and 41,690	E.g., automated, cartridge-based, multiplexing capacity
Alinity	Plasma/CSF	Chemiluminescent microparticle immunoassay	The Alinity i-system	6.1–42,000	6.1	No dilution		REF 04W1720	E.g., fully-automated, high-throughput
MSD	Plasma/CSF	Electrochemiluminescence	MESO QuickPlex SQ®	0.309–683 ^a (0.62–1,360 when including 2× plasma sample dilution)	0.309	Plasma 2× CSF 20×	Plasma Low: %CV _r : 12.0 %CV _{Rw} : N/A High: %CV _r : 12.0 %CV _{Rw} : N/A CSF %CV _r : 6.9 %CV _{Rw} : N/A High: %CV _r : 3.9 %CV _{Rw} : N/A %CV _r : N/A %CV _{Rw} : 18.0	K00S0157	E.g., multiplexing capability
ELISA	CSF	Enzyme-linked immunoassay, colorimetric readout	Sunrise microplate reader (Tecan Trading AG)	70–8,000	70	No dilution		None (in-house method)	E.g., in-house developed assay

CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; LLoQ, lower limit of quantification; MSD, meso scale discovery; Simoa, single molecule array; REF, reference; %CV_r, repeatability; %CV_{Rw}, intermediate precision; N/A, not available. ^aSimoa, reported LLoQ obtained from manufacturer documentation; Ella, LLoQ determined in-house; Alinity, LLoQ obtained from manufacturer documentation; MSD, LLoQ determined in-house; ELISA, LLoQ determined in-house. ^bFor the Alinity assay, routine quality control samples were measured before and after each run, with %CVs, consistently below 10 %, in accordance with our standard operating procedure for fully automated immunoassays. If the %CVs, exceeded this threshold, the run was stopped.

Table 2: Summary of method comparison for plasma and CSF GFAP assays: correlation, Bland–Altman analysis, and Passing–Bablok regression.

Comparison	Spearman (r, p-Value)	Mean difference (95 % CI), pg/mL	Bland–Altman		Passing–Bablok regression	
			Limits of agreement (95 % CI)	% mean difference (95 % CI)	Limits of agreement (95 % CI)	Observed bias (Slope, intercept)
Plasma						
Alinity vs. Ella	0.839, p<0.0001	25.6 (21.5–29.6)	7.1 (0.02–14.2) to 44.1 (37.0–51.1)	113.8 % (107.6–119.9 %)	85.8 % (75.1–96.5 %) to 141.7 % (131.0–152.4 %)	3.778 (2.693–5.515), 0.696 (–13.466–8.651) Proportional
Simoa vs. Alinity	0.927, p<0.0001	164.9 (129.7–200.1)	–47.9 (–108.5–12.8) to 377.7 (317.1–438.4)	149.6 % (146.7–152.6 %)	131.7 % (126.6–136.8 %) to 167.5 % (162.4–172.6 %)	0.1268 (0.1039–0.1479), 2.2134 (–0.2616–5.7071) Proportional
Simoa vs. MSD	0.914, p<0.0001	–452.0 (–536.4–367.5)	–926.2 (–1071.9–780.5) to 22.3 (–123.5–168.0)	–107.5 % (–111.9 %–103.0 %)	–132.5 % (–140.2 %–123.8 %) to –82.4 % (–90.1 %–74.7 %)	3.5509 (3.1935–3.9167), –36.8 (–87.9–19.2) Proportional
Simoa vs. Ella	0.827, p<0.0001	271.4 (233.9–308.8)	7.7 (–56.8–72.1) to 535.1 (470.7–599.5)	185.7 % (184.6–186.7 %)	178.3 % (176.5–180.1 %) to 193.0 % (191.2–194.8 %)	36.031 (30.923–43.615), –64.712 (–126.731 to –20.284) Proportional and systematic
CSF						
ELISA vs. Alinity	0.937, p<0.0001	–1762.9 (–2158.0–1367.8)	–4429.6 (–5109.5–3749.7) to 903.8 (223.9–1583.7)	–117.0 % (–122.4 %–111.7 %)	–153.2 % (–162.4 %–144.0 %) to –80.9 % (–90.1 %–71.6 %)	4.0732 (3.7084– 4.4810), –100.47 (–220.81–4.67) Proportional and systematic
ELISA vs. MSD	0.949, p<0.0001	–33894.2 (–43398.7–24389.7)	–87284.6(–103689.1–70880.0)	–195.5 % (–195.8 % to –195.1 %)	–197.5 % (–198.1 % to –196.9 %) to –193.4 % (–194.0 % to –192.8 %)	91.85 (80.65– 106.10), –820.19 (–3,421.94 to 1486.04) Proportional
ELISA vs. Ella	0.958, p<0.0001	–55.0 (–89.4–20.6)	–294.6 (–353.7–235.4) to 184.6 (125.4–243.7)	–21.3 % (–30.9 %–11.6 %)	–88.6 % (–105.2 %–72.0 %) to 46.0 % (29.4–62.6 %)	0.9698 (0.9351–1.0158), 45.18 (26.65–58.51) Systematic

CI, confidence interval; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; MSD, meso scale discovery; Simoa, single molecule array; r, spearman correlation coefficient. Absolute concentration differences are presented as mean difference (95 % CI) in ng/L, and relative differences are presented as % mean difference (95 % CI). The corresponding lower and upper limits of agreement are shown alongside each mean difference.

concentrations compared with Ella. Passing–Bablok regression revealed proportional bias only, with a slope of 3.778 (95 % CI: 2.693–5.515) and an intercept of 0.696 (95 % CI: –13.466 to 8.651), indicating that the difference between methods increased with higher GFAP concentrations (Table 2, Figure 1A).

Similarly, a robust correlation was observed between the Simoa and Alinity assays ($r_s=0.927$, $p<0.0001$). Bland–Altman analysis showed a mean difference of 149.6 % (95 % CI: 146.7–152.6 %), with limits of agreement from 131.7 % (95 % CI: 126.6–136.8 %) to 167.5 % (95 % CI: 162.4–172.6 %), reflecting that Simoa consistently reported higher GFAP

concentrations than Alinity. Passing–Bablok regression revealed proportional bias only, with a slope of 0.1268 (95 % CI: 0.1039–0.1479) and an intercept of 2.2134 (95 % CI: –0.2616–5.7071), indicating that the discrepancy between the assays increased at higher GFAP concentrations (Table 2, Figure 1B).

The comparison between Simoa and MSD assays demonstrated a strong correlation ($r_s=0.914$, $p<0.0001$). Bland–Altman analysis indicated a mean difference of –107.5 % (95 % CI: –111.9 %–103.0 %), with limits of agreement ranging from –132.5 % (95 % CI: –140.2 %–123.8 %) to –82.4 % (95 % CI: –90.1 %–74.7 %), demonstrating that Simoa systematically

Plasma A - Alinity vs Ella
B - Simoa vs Alinity

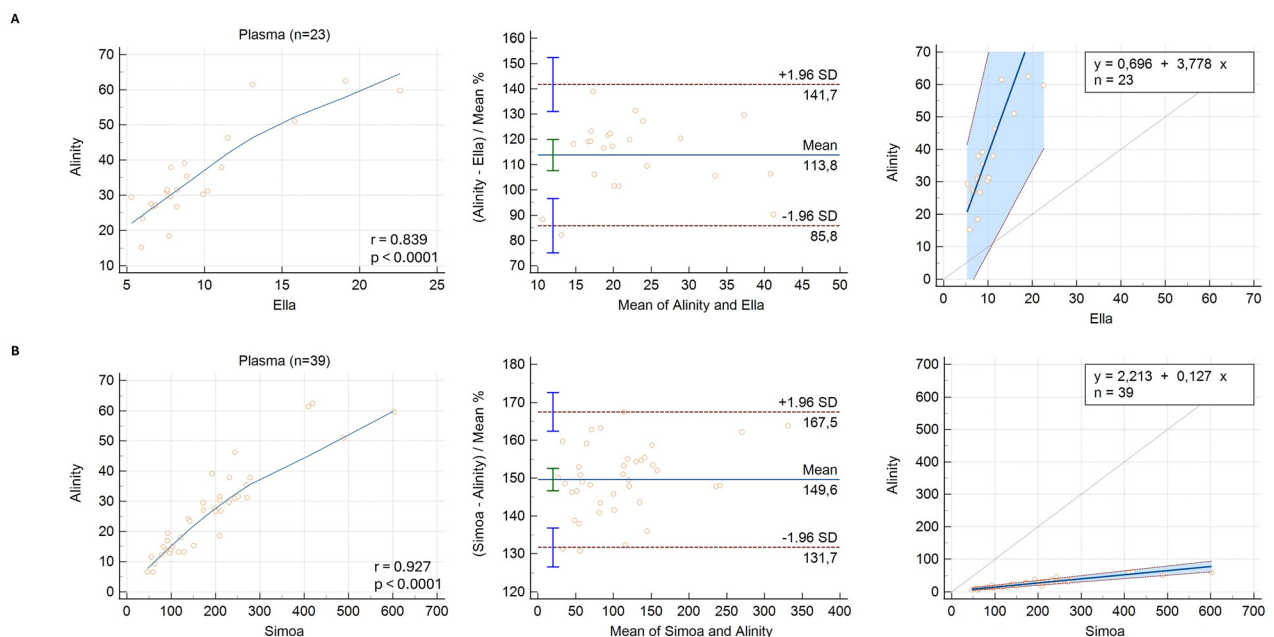


Figure 1: Method comparison of plasma assays: Alinity vs. Ella and Alinity vs. Simoa each row shows the correlation plot (left), Bland–Altman plot (middle), and Passing–Bablok regression (right) for each pairwise comparison. (A) Left: Correlation plot of plasma GFAP concentrations measured by Ella (x-axis) and Abbott Alinity (y-axis) assays, with values expressed in ng/L. The plot demonstrates a strong correlation ($r=0.839$, $p<0.0001$). A locally estimated scatterplot smoothing (LOESS) curve with an 80 % span is applied to the trend line to enhance the visualization of the data distribution. (A) Middle: Bland–Altman plot showing the percentage differences (y-axis) between Abbott Alinity and Ella assays against their mean concentrations (x-axis). The solid blue line represents the mean bias, and the dashed brown lines indicate the limits of agreement ($\pm 1.96SD$). The blue error bars represent the 95 % confidence intervals (CIs) for the limits of agreement, while the green error bar shows the 95 % CI for the mean bias. The mean difference of 113.8 % (95 % CI: 107.6–119.9 %) is shown, with limits of agreement ranging from 85.8 % (95 % CI: 75.1–96.5 %) to 141.7 % (95 % CI: 131.0–152.4 %). (A) Right: Passing–Bablok regression plot with GFAP concentrations from Ella on the x-axis and Abbott Alinity on the y-axis, demonstrating proportional bias with a slope of 3.778 (95 % CI: 2.693–5.515) and an intercept of 0.696 (95 % CI: –13.466–8.651). The solid blue line represents the fitted regression line, while the shaded blue area indicates the confidence interval and the dashed brown line represents the identity line ($y=x$). (B) Left: Correlation plot of GFAP concentrations measured by Simoa (x-axis) and Abbott Alinity (y-axis) assays, with values expressed in ng/L. The plot demonstrates a robust correlation ($r=0.927$, $p<0.0001$). A locally estimated scatterplot smoothing (LOESS) curve with an 80 % span is applied to the trend line to better visualize the data distribution. (B) Middle: Bland–Altman plot showing the percentage differences (y-axis) between Simoa and Abbott Alinity assays against their mean concentrations (x-axis). The solid blue line represents the mean bias, and the dashed brown lines indicate the limits of agreement ($\pm 1.96SD$). The blue error bars represent the 95 % confidence intervals (CIs) for the limits of agreement, while the green error bar shows the 95 % CI for the mean bias. The mean difference of 149.6 % (95 % CI: 146.7–152.6 %) is highlighted, with limits of agreement from 131.7 % (95 % CI: 126.6–136.8 %) to 167.5 % (95 % CI: 162.4–172.6 %). (B) Right: Passing–Bablok regression plot with GFAP concentrations from Simoa on the x-axis and Alinity on the y-axis, highlighting proportional bias with a slope of 0.1268 (95 % CI: 0.1039–0.1479) and an intercept of 2.2134 (95 % CI: –0.2616–5.7071). The solid blue line represents the fitted regression line, while the shaded blue area indicates the confidence interval and the dashed brown line represents the identity line ($y=x$).

reported lower GFAP concentrations compared with MSD. Passing–Bablok regression identified a significant proportional bias but no systematic bias, with a slope of 3.5509 (95 % CI: 3.1935–3.9167) and an intercept of -36.8 (95 % CI: -87.9 – 19.2), as the difference between assays became more pronounced at higher GFAP concentrations (Table 2, Figure 2A).

Lastly, the comparison between the Simoa and Ella assays revealed a strong correlation ($r_s=0.827$, $p<0.0001$). Bland–Altman analysis reported a mean difference of 185.7 % (95 % CI: 184.6–186.7 %), with limits of agreement ranging from 178.3 % (95 % CI: 176.5–180.1 %) to 193.0 % (95 % CI: 191.2–194.8 %), indicating that Simoa consistently overestimated GFAP concentrations compared with Ella.

Passing–Bablok regression identified proportional and systematic differences, with a slope of 36.031 (95 % CI: 30.923–43.615) and an intercept of -64.712 (95 % CI: -126.731 to -20.284), suggesting that the discrepancy between assays increased with concentration and was present across the entire range (Table 2, Figure 2B).

Method comparison of CSF assays

A strong correlation was observed between the ELISA and Alinity assays in CSF samples ($r_s=0.937$, $p<0.0001$). Bland–Altman analysis indicated a mean difference of -117.0 %

Plasma A – Simoa vs MSD
B – Simoa vs Ella

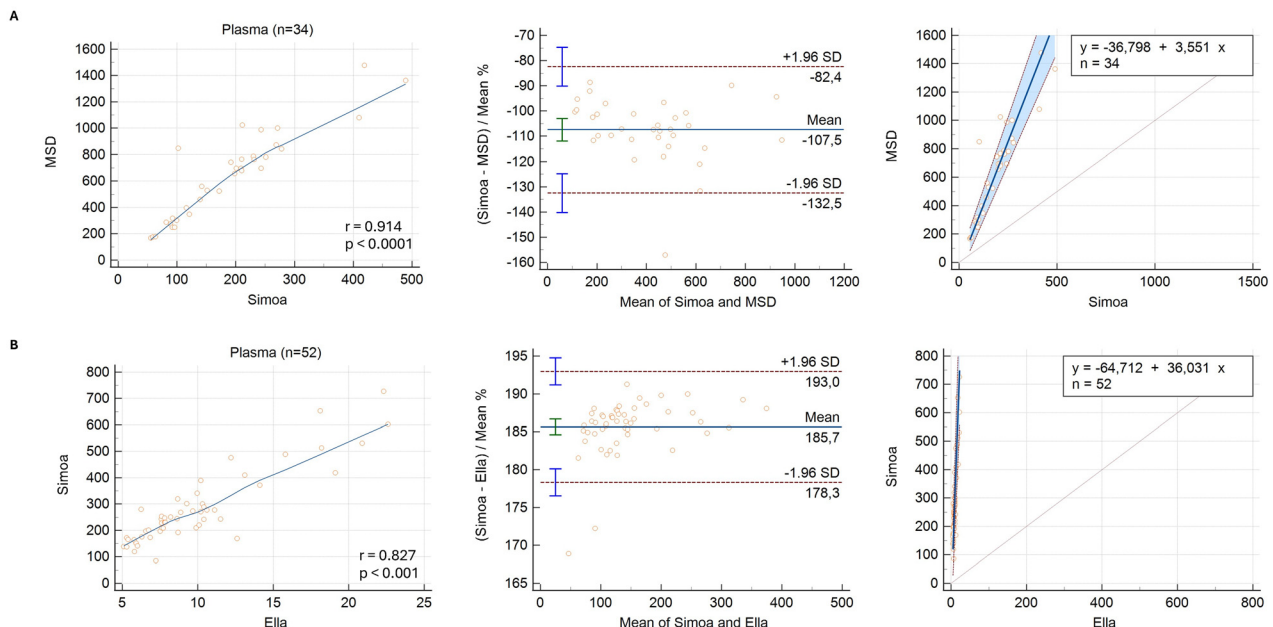


Figure 2: Method comparison of plasma assays: Simoa vs. MSD and Simoa vs. Ella. Each row shows the correlation plot (left), Bland–Altman plot (middle), and Passing–Bablok regression (right) for each pairwise comparison. (A) Left: Correlation plot of GFAP concentrations measured by Simoa (x-axis) and MSD (y-axis) assays, with values expressed in ng/L. The plot demonstrates a strong correlation ($r=0.914$, $p<0.0001$). A locally estimated scatterplot smoothing (LOESS) curve with an 80 % span is applied to the trend line to enhance visualization of the data distribution. (A) Middle: Bland–Altman plot showing the percentage differences (y-axis) between simoa and MSD assays against their mean concentrations (x-axis). The solid blue line represents the mean bias, and the dashed brown lines indicate the limits of agreement ($\pm 1.96SD$). The blue error bars represent the 95 % confidence intervals (CIs) for the limits of agreement, while the green error bar shows the 95 % CI for the mean bias. The mean difference of -107.5 % (95 % CI: -111.9 %– -103.0 %) is presented, with limits of agreement from -132.5 % (95 % CI: -140.2 %– -123.8 %) to -82.4 % (95 % CI: -90.1 %– -74.7 %). (A) Right: Passing–Bablok regression plot with GFAP concentrations from Simoa on the x-axis and MSD on the y-axis, indicating proportional bias with a slope of 3.5509 (95 % CI: 3.1935–3.9167) and an intercept of -36.8 (95 % CI: -87.9 – 19.2). The solid blue line represents the fitted regression line, while the shaded blue area indicates the confidence interval and the dashed brown line represents the identity line ($y=x$). (B) Left: Correlation plot of GFAP concentrations measured by Ella (x-axis) and Simoa (y-axis) assays, with values expressed in ng/L. The plot demonstrates a strong correlation ($r=0.827$, $p<0.0001$). A locally estimated scatterplot smoothing (LOESS) curve with an 80 % span is applied to the trend line to enhance visualization of the data distribution. (B) Middle: Bland–Altman plot showing the percentage differences (y-axis) between Simoa and Ella assays against their mean concentrations (x-axis). The solid blue line represents the mean bias, and the dashed brown lines indicate the limits of agreement ($\pm 1.96SD$). The blue error bars represent the 95 % confidence intervals (CIs) for the limits of agreement, while the green error bar shows the 95 % CI for the mean bias. The mean difference of 185.7 % (95 % CI: 184.6 – 186.7 %) is shown, with limits of agreement from 178.3 % (95 % CI: 176.5 – 180.1 %) to 193.0 % (95 % CI: 191.2 – 194.8 %). (B) Right: Passing–Bablok regression plot with GFAP concentrations from Ella on the x-axis and Simoa on the y-axis, highlighting proportional bias with a slope of 36.031 (95 % CI: 30.923–43.615) and an intercept of -64.712 (95 % CI: -126.731 – -20.284). The solid blue line represents the fitted regression line, while the shaded blue area indicates the confidence interval and the dashed brown line represents the identity line ($y=x$).

(95 % CI: -122.4 %—111.7 %), with limits of agreement ranging from -153.2 % (95 % CI: -162.4 %—144.0 %) to -80.9 % (95 % CI: -90.1 %—71.6 %), showing that ELISA reported lower GFAP concentrations than Alinity. Passing-Bablok regression revealed both systematic and proportional biases, with a slope of 4.0732 (95 % CI: 3.7084–4.4810) and an intercept of -100.47 (95 % CI: -220.81–4.67), indicating that Alinity measurements were consistently higher than ELISA, with the difference becoming more pronounced at higher GFAP concentrations (Table 2, Figure 3A).

Similarly, a strong correlation was observed between the ELISA and MSD assays in CSF samples ($r_s=0.949$, $p<0.0001$). Bland-Altman analysis showed a mean difference of -195.5 % (95 % CI: -195.8 %—195.1 %), with limits of agreement ranging from -197.5 % (95 % CI: -198.1 %—196.9 %) to -193.4 % (95 % CI: -194.0 %—192.8 %), with ELISA underestimating GFAP levels relative to MSD. Passing-Bablok regression analysis further confirmed a substantial proportional bias, with a slope of 91.85 (95 % CI: 80.65–106.10) and an intercept of -820.19 (95 % CI: -3421.94–1486.04), indicating that MSD measurements were disproportionately higher than ELISA as GFAP concentrations increased (Table 2, Figure 3B).

Lastly, a strong correlation was observed between the ELISA and Ella assays in CSF samples ($r_s=0.958$, $p<0.0001$). Bland-Altman analysis indicated a mean difference of -21.3 % (95 % CI: -30.9 %—11.6 %), with limits of agreement ranging from -88.6 % (95 % CI: -105.2 %—72.0 %) to 46.0 % (95 % CI: 29.4–62.6 %). Passing-Bablok regression analysis showed only systematic bias, with a slope of 0.9698 (95 % CI: 0.9351–1.0158) and an intercept of 45.18 (95 % CI: 26.65–58.51) (Table 2, Figure 3C).

The regression equations obtained from the Passing-Bablok regression analysis are presented in Table 3.

Discussion

In this study, we compared four different GFAP immunoassays for plasma and four for CSF, conducting pairwise comparisons using distinct sample sets. Although Spearman's r ranged from 0.827 to 0.958 across assays, our analysis revealed substantial method-dependent biases in several comparisons. Some assays exhibited a systematic bias, characterized by a consistent offset across all concentrations, whereas others demonstrated a proportional bias, where discrepancies increased with higher GFAP levels. Additionally, several comparisons revealed the presence of both systematic and proportional biases, indicating assay-specific differences in GFAP quantification.

To better understand the practical implications of these findings, we examined the extent and nature of these biases

in both plasma and CSF assay comparisons. Among the plasma GFAP assay comparisons, both systematic and proportional biases were observed only between Simoa and Ella, while the remaining comparisons showed proportional bias alone. These discrepancies have important implications for biomarker standardization and clinical implementation. In particular, the lack of agreement in absolute concentrations – most evident in comparisons such as Simoa vs. Alinity and Simoa vs. MSD – suggests that differences in antibody specificity, epitope recognition, assay design (e.g., two-step vs. three-step protocols), detection technologies, and calibration strategies likely contribute to the observed variability. For instance, proportional biases that became more pronounced at higher GFAP concentrations highlight disparities in analytical sensitivity and dynamic range, complicating the direct interchangeability of results across assays. Simoa generally reported higher absolute concentrations than other assays – except in the comparison with MSD, where MSD reported higher values – highlighting variability even among high-sensitivity platforms. In CSF, assay comparisons demonstrated that ELISA consistently underestimated GFAP levels relative to Alinity, MSD, and Ella, with the greatest discrepancy observed in ELISA vs. MSD and the smallest in ELISA vs. Ella. Both systematic and proportional biases were present, reinforcing that assay-specific differences persist across matrices. These findings collectively emphasize that GFAP measurements are not directly interchangeable between platforms without appropriate bias correction, which is essential for consistent interpretation in clinical and research contexts.

Given these observed discrepancies, regression-based harmonization might serve as a practical interim solution. The regression equations derived from our method comparison using Passing-Bablok analysis may serve as provisional tools to translate values between assays in the absence of standardized calibration for GFAP. In this study, the analysis revealed both proportional and fixed biases, and the resulting equations could facilitate approximate cross-assay comparisons under controlled conditions. However, the applicability of these equations must be approached with caution. Their validity is inherently dependent on the distribution and range of GFAP concentrations within the study population; for instance, age-related elevations in GFAP, particularly in older individuals, may introduce variability that limits the generalizability of such transformations. Additionally, our analyses were conducted exclusively using plasma samples, meaning these equations are not directly transferable to other matrices such as serum or lithium heparin plasma without further validation. Importantly, due to differences in antibody specificities and detection technologies among platforms, assay results may not be directly

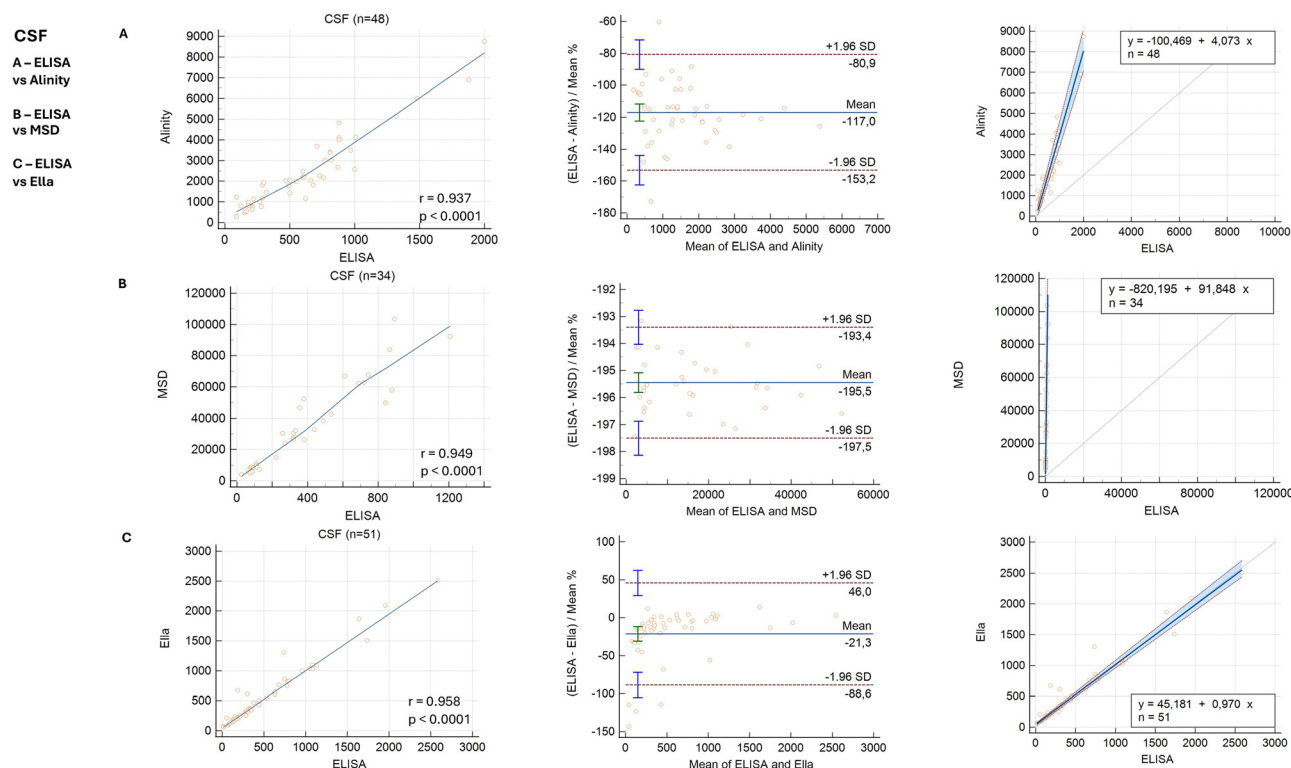


Figure 3: Method comparison of CSF assays: ELISA vs. Alinity, ELISA vs. MSD, and ELISA vs. Ella each row shows the correlation plot (left), Bland–Altman plot (middle), and Passing–Bablok regression (right) for each pairwise comparison. (A) Left: In the correlation plot, GFAP concentrations measured by ELISA (x-axis) and alinity (y-axis) are expressed in ng/L, demonstrating a strong correlation ($r=0.937$, $p<0.0001$). A locally estimated scatterplot smoothing (LOESS) curve with an 80 % span is applied to the trend line to improve visualization of the data distribution. (A) Middle: Bland–Altman plot showing the percentage differences (y-axis) between ELISA and Alinity assays against their mean concentrations (x-axis). The solid blue line represents the mean bias, and the dashed brown lines indicate the limits of agreement ($\pm 1.96SD$). The blue error bars represent the 95 % confidence intervals (CIs) for the limits of agreement, while the green error bar shows the 95 % CI for the mean bias. The mean difference of -117.0 (95 % CI: -122.4% – -111.7%) is shown, with limits of agreement from -153.2 (95 % CI: -162.4% – -144.0%) to -80.9 (95 % CI: -90.1% – -71.6%). (A) Right: Passing–Bablok regression plot with GFAP concentrations from ELISA on the x-axis and Alinity on the y-axis, highlighting proportional bias with a slope of 4.0732 (95 % CI: 3.7084 – 4.4810) and an intercept of -100.47 (95 % CI: -220.81 – -4.67). The solid blue line represents the fitted regression line, while the shaded blue area indicates the confidence interval and the dashed brown line represents the identity line ($y=x$). (B) Left: In the correlation plot, GFAP concentrations measured by ELISA (x-axis) and MSD (y-axis) are expressed in ng/L, demonstrating a strong correlation ($r=0.949$, $p<0.0001$). A locally estimated scatterplot smoothing (LOESS) curve with an 80 % span is applied to the trend line to better visualize the data distribution. (B) Middle: Bland–Altman plot showing the percentage differences (y-axis) between ELISA and MSD assays against their mean concentrations (x-axis). The solid blue line represents the mean bias, and the dashed brown lines indicate the limits of agreement ($\pm 1.96SD$). The blue error bars represent the 95 % confidence intervals (CIs) for the limits of agreement, while the green error bar shows the 95 % CI for the mean bias. The mean difference of -195.5 (95 % CI: -195.8% – -195.1%), is shown, with limits of agreement from -197.5 (95 % CI: -198.1% – -196.9%) to -193.4 (95 % CI: -194.0% – -192.8%). (B) Right: Passing–Bablok regression plot with GFAP concentrations from ELISA on the x-axis and MSD on the y-axis, highlighting proportional bias with a slope of 91.85 (95 % CI: 80.65 – 106.10) and an intercept of -820.19 (95 % CI: -3421.94 – 1486.04). The solid blue line represents the fitted regression line, while the shaded blue area indicates the confidence interval and the dashed brown line represents the identity line ($y=x$). (C) Left: In the correlation plot, GFAP concentrations measured by ELISA (x-axis) and Ella (y-axis) are expressed in ng/L, demonstrating a strong positive correlation ($r=0.958$, $p<0.0001$). A locally estimated scatterplot smoothing (LOESS) curve with an 80 % span is applied to the trend line to better visualize the data distribution. (C) Middle: Bland–Altman plot showing the percentage differences (y-axis) between ELISA and Ella assays against their mean concentrations (x-axis). The solid blue line represents the mean bias, and the dashed brown lines indicate the limits of agreement ($\pm 1.96SD$). The blue error bars represent the 95 % confidence intervals (CIs) for the limits of agreement, while the green error bar shows the 95 % CI for the mean bias. The mean difference of -21.3 (95 % CI: -30.9% – -11.6%) is shown, with limits of agreement from -88.6 (-105.2% – -72.0%) to 46.0 (29.4 – 62.6%). (C) Right: Passing–Bablok regression plot with GFAP concentrations from ELISA on the x-axis and Ella on the y-axis, highlighting proportional bias with a slope of 0.9698 (95 % CI: 0.9351 – 1.0158) and an intercept of 45.18 (95 % CI: 26.65 – 58.51). The solid blue line represents the fitted regression line, while the shaded blue area indicates the confidence interval and the dashed brown line represents the identity line ($y=x$).

interchangeable. This lack of commutability increases the risk of misclassification, especially near clinically significant thresholds where even minor discrepancies can affect

diagnostic or prognostic decisions. Therefore, we advise that these regression models be used judiciously and primarily in research settings. Until certified reference material (CRM)

Table 3: Passing–Bablok regression equations for each pairwise comparison of plasma and CSF GFAP assays.

Pairwise comparison	Regression equation
Plasma	
Alinity vs. Ella	Alinity=0.696+3.778*Ella
Simoa vs. Alinity	Alinity=2.213+0.127*Simoa
Simoa vs. MSD	MSD=−36.798+3.551*Simoa
Simoa vs. Ella	Simoa=−64.712+36.031*Ella
CSF	
ELISA vs. Alinity	Alinity=−100.469+4.073*ELISA
ELISA vs. MSD	MSD=−820.195+91.848*ELISA
ELISA vs. Ella	Ella=45.181+0.970*ELISA

CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; MSD, meso scale discovery; Simoa, single molecule array.

and traceable calibrators are established for GFAP, regression-based harmonization should be considered a temporary and context-dependent solution. For clinical applications, particularly where established cutoffs exist (e.g., in mild TBI), we recommend using assay- and matrix-specific reference values.

These considerations further underscore the complexities involved in achieving cross-platform consistency and highlight the influence of both technical and biological factors. As noted in the Introduction, several factors – such as differences in antibody selection, assay design, and calibrators – pose challenges for cross-platform standardization of GFAP measurements. Notably, in our study, the strongest correlation in plasma pairwise comparisons was observed between the Simoa and Alinity platforms (Spearman’s $r=0.927$), both of which reportedly use antibodies derived from Banyan Biomarkers. This finding supports the idea that using antibodies from the same source may contribute to improved analytical concordance. However, the correlation was not perfect, and lower correlations were observed for other pairwise comparisons, such as Alinity vs. Ella ($r=0.839$) and Simoa vs. Ella ($r=0.827$). These discrepancies suggest that, beyond antibody origin, other technical differences – such as assay configuration (e.g., two-step vs. three-step), calibrator materials, and assay platforms (e.g., Simoa vs. chemiluminescent microparticle immunoassay) – may influence the overall agreement. In contrast, GFAP concentrations measured in CSF showed strong correlations across all assays, which may be attributed to the simpler and more homogeneous nature of the CSF matrix. One plausible explanation is that GFAP in CSF exists in a more uniform form, allowing different antibody pairs to recognize the target epitope with similar efficiency. Additionally, the lower total protein content and fewer interfering substances in CSF likely contribute to the improved assay

concordance observed in this matrix. Altogether, these findings suggest that the biological matrix itself plays a significant role in determining inter-assay comparability. They also highlight the need for a multifaceted approach to assay harmonization, including the use of standardized calibrators and potentially matrix-specific validation strategies.

Our findings build on prior developments in GFAP quantification, which have evolved from initial ELISA-based measurements in CSF [10, 18] to the emergence of various immunoassay platforms applicable to both CSF and blood [9, 10, 12, 16]. These advances have enabled detection across a range of neurological conditions, including TBI [23], neuro-immunological disorders [24], AD [25], and glioblastoma multiforme [26, 27]. More recently, fully automated platforms [15, 29] and point-of-care technologies [30] have expanded the clinical potential of GFAP testing. However, despite this progress, direct comparisons between assays remain limited, and cross-platform differences in absolute GFAP concentrations are increasingly recognized. In a recent study [28], Fazeli et al. compared a newly developed second-generation microfluidic immunoassay (Ella) with Simoa and a homebrew Ella assay for serum samples, reporting a substantial difference in absolute GFAP concentrations, with markedly higher levels detected by the Simoa assay. Consistent with their findings, we also observed a significant difference in absolute GFAP concentrations between Simoa and Ella, despite the two assays showing a strong correlation in plasma samples.

Despite overall assay concordance, the presence of notable systematic and proportional biases indicates that these methods are not directly interchangeable. This lack of interchangeability highlights the urgent need for standardization across GFAP assays. Establishing standardized reference materials and aligning calibration strategies across assays will be essential to improve comparability and support the clinical implementation of GFAP as a biomarker. A notable example of successful biomarker standardization is the effort to harmonize CSF amyloid-beta 1–42 (A β 42) measurements [31]. To minimize variability between different assays, the European Commission’s Joint Research Centre (JRC), in collaboration with the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), established CRMs specifically for CSF A β 42 [31]. Applying a comparable strategy to GFAP, including the creation of CRMs and assay recalibration, could be instrumental in mitigating systematic and proportional biases across measurement platforms.

This study has several strengths and limitations. One limitation is that, due to the availability of the assays included in this comprehensive method comparison and constraints related to sample volume, we were unable to use the same samples for all pairwise comparisons. However, we

addressed this by selecting similar samples with comparable concentration ranges, and this approach was statistically evaluated. While the CLSI EP09C guidelines recommend a minimum of 40 samples for method comparison studies, some pairwise comparisons in our study included fewer samples. The lowest sample size used was 23, which we believe remains acceptable, as our dataset adequately covered the measurement range of the assays tested. Another limitation is the absence of a direct comparison between the Alinity I TBI and Vidas® TBI assays [32], both of which have received FDA approval and CE marking for the evaluation of mTBI. While these assays are increasingly used in clinical practice, logistical and technical constraints prevented their inclusion in the present analysis. Future studies directly comparing these two commercially available platforms would be valuable for assessing their analytical agreement and clinical applicability. Lastly, we were unable to compare paired CSF and plasma samples from the same individuals. As CSF GFAP is highly sensitive to freeze–thaw cycles, such a comparison ideally requires freshly collected samples, which were not available in sufficient quantity for this study. On the other hand, a major strength of this study is that, for the first time, multiple commercially available plasma and CSF GFAP assays were directly compared, revealing substantial biases in absolute GFAP concentrations between different platforms. These findings may contribute to future efforts in developing standardized reference materials, given the observed discrepancies among assays. Although we were unable to use identical sample sets across all comparisons, the distinct sets we analyzed reflect the types of clinical samples commonly received in routine laboratory practice for the measurement of other neurobiomarkers, such as neurofilament light (NfL).

In conclusion, our findings demonstrate that commercially available plasma and CSF GFAP assays correlate strongly but exhibit both systematic and proportional biases, emphasizing the need for standardization efforts. Harmonization across different assays is essential to ensure reliable longitudinal tracking of GFAP concentrations in clinical and research settings, particularly when transitioning between different platforms. The choice of platform depends on multiple factors, including analytical performance, sensitivity, sample volume requirements, and laboratory infrastructure – areas that warrant further investigation. Until cross-platform harmonization is achieved, assay-specific reference ranges or transformation models would be necessary. Future studies should focus on developing CRMs and standardized calibration approaches to improve the comparability of GFAP assays.

Research ethics: The collection of anonymized samples at the Clinical Chemistry Laboratory, Sahlgrenska University

Hospital, was carried out in compliance with the ethical guidelines approved by the Ethics Committee at the University of Gothenburg (EPN140811).

Informed consent: Not applicable.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Use of Large Language Models, AI and Machine Learning Tools: None declared.

Conflict of interest: KB has served as a consultant and at advisory boards for Abbvie, AC Immune, ALZPath, AriBio, BioArctic, Biogen, Eisai, Lilly, Moleac Pte. Ltd, Neurimmune, Novartis, Ono Pharma, Prothena, Roche Diagnostics, Sanofi and Siemens Healthineers; has served at data monitoring committees for Julius Clinical and Novartis; has given lectures, produced educational materials and participated in educational programs for AC Immune, Biogen, Celdara Medical, Eisai and Roche Diagnostics; and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Acumen, Alector, Alzinova, ALZPath, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Optoceutics, Passage Bio, Pinteon Therapeutics, Prothena, Red Abbey Labs, reMYND, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

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Data availability: The data supporting the findings of this study may be shared with qualified academic investigators for the purpose of result replication, upon reasonable request to the corresponding author and under a material transfer agreement.

References

- Hol EM, Pekny M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate filament system in diseases of the central nervous system. *Curr Opin Cell Biol* 2015;32:121–30.
- Middeldorp J, Hol E. GFAP in health and disease. *Prog Neurobiol* 2011; 93:421–43.
- Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat Rev Neurol* 2013;9:201–10.
- Petzold A. Glial fibrillary acidic protein is a body fluid biomarker for glial pathology in human disease. *Brain Res* 2015;1600:17–31.
- Benedet AL, Milà-Alomà M, Vrillon A, Ashton NJ, Pascoal TA, Lussier F, et al. Differences between plasma and cerebrospinal fluid glial fibrillary acidic protein levels across the Alzheimer disease continuum. *JAMA Neurol* 2021;78:1471–83.
- Pereira JB, Janelidze S, Smith R, Mattsson-Carlsson N, Palmqvist S, Teunissen CE, et al. Plasma GFAP is an early marker of amyloid- β but not tau pathology in Alzheimer's disease. *Brain* 2021;144:3505–16.
- Simrén J, Weninger H, Brum WS, Khalil S, Benedet AL, Blennow K, et al. Differences between blood and cerebrospinal fluid glial fibrillary acidic protein levels: the effect of sample stability. *Alzheimer's Dement* 2022; 18:1988–92.
- Chatterjee P, Doré V, Pedrini S, Krishnadas N, Thota R, Bourgeat P, et al. Plasma glial fibrillary acidic protein is associated with 18F-SMBT-1 PET: two putative astrocyte reactivity biomarkers for Alzheimer's disease. *J Alzheimers Dis* 2023;92:615–28.
- Arslan B, Zetterberg H, Ashton NJ. Blood-based biomarkers in Alzheimer's disease—moving towards a new era of diagnostics. *Clin Chem Lab Med* 2024;62:1063–9.
- Petzold A, Keir G, Green A, Giovannoni G, Thompson E. An ELISA for glial fibrillary acidic protein. *J Immunol Methods* 2004;287:169–77.
- Bogoslovsky T, Wilson D, Chen Y, Hanlon D, Gill J, Jeromin A, et al. Increases of plasma levels of glial fibrillary acidic protein, tau, and amyloid β up to 90 days after traumatic brain injury. *J Neurotrauma* 2017;34:66–73.
- Fazeli B, Huss A, Gómez de San José N, Otto M, Tuman H, Halbgebauer S. Development of an ultrasensitive microfluidic assay for the analysis of Glial fibrillary acidic protein (GFAP) in blood. *Front Mol Biosci* 2023;10:1175230.
- Button EB, Cheng WH, Barron C, Cheung H, Bashir A, Cooper J, et al. Development of a novel, sensitive translational immunoassay to detect plasma glial fibrillary acidic protein (GFAP) after murine traumatic brain injury. *Alzheimers Res Ther* 2021;13:1–27.
- Menditto VG, Moretti M, Babini L, Mattioli A, Giuliani AR, Fratini M, et al. Minor head injury in anticoagulated patients: performance of biomarkers S100B, NSE, GFAP, UCH-L1 and Alinity TBI in the detection of intracranial injury. A prospective observational study. *Clin Chem Lab Med* 2024;62:1376–82.
- Kamada J, Hamanaka T, Oshimo A, Sato H, Nishii T, Fujita M, et al. Glial fibrillary acidic protein's usefulness as an astrocyte biomarker using the fully automated LUMIPULSE® system. *Diagnostics* 2024;14:2520.
- Abdelhak A, Huss A, Kassubek J, Tuman H, Otto M. Serum GFAP as a biomarker for disease severity in multiple sclerosis. *Sci Rep* 2018;8: 14798.
- Thelin E, Al Nimer F, Frostell A, Zetterberg H, Blennow K, Nyström H, et al. A serum protein biomarker panel improves outcome prediction in human traumatic brain injury. *J Neurotrauma* 2019;36:2850–62.
- Rosengren LE, Wikkelsø C, Hagberg L. A sensitive ELISA for glial fibrillary acidic protein: application in CSF of adults. *J Neurosci Methods* 1994;51:197–204.
- Tukey JW. *Exploratory data analysis*, 18th ed. Addison-Wesley Publishing Company; 1977:688 p.
- Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res* 1999;8:135–60.
- Passing H, Bablok W. Comparison of several regression procedures for method comparison studies and determination of sample sizes application of linear regression procedures for method comparison studies in clinical chemistry, part II. *J Clin Chem Clin Biochem* 1984;22: 431–45.
- Bilic-Zulle L. Comparison of methods: Passing and Bablok regression. *Biochem Med* 2011;21:49–52.
- Fraser DD, Close TE, Rose KL, Ward R, Mehl M, Farrell C, et al. Severe traumatic brain injury in children elevates glial fibrillary acidic protein in cerebrospinal fluid and serum. *Pediatr Crit Care Med* 2011;12:319–24.
- Kassubek R, Gorges M, Schocke M, Hagenston VA, Huss A, Ludolph AC, et al. GFAP in early multiple sclerosis: a biomarker for inflammation. *Neurosci Lett* 2017;657:166–70.
- Fukuyama R, Izumoto T, Fushiki S. The cerebrospinal fluid level of glial fibrillary acidic protein is increased in cerebrospinal fluid from Alzheimer's disease patients and correlates with severity of dementia. *Eur Neurol* 2001;46:35–8.
- Jung C, Foerch C, Schänzer A, Heck A, Plate K, Seifert V, et al. Serum GFAP is a diagnostic marker for glioblastoma multiforme. *Brain* 2007; 130:3336–41.
- van Asperen JV, Fedorushkova DM, Robe PA, Hol EM. Investigation of glial fibrillary acidic protein (GFAP) in body fluids as a potential biomarker for glioma: a systematic review and meta-analysis. *Biomarkers* 2022;27:1–12.
- Fazeli B, Gómez de San José N, Jesse S, Senel M, Oeckl P, Erhart DK, et al. Quantification of blood glial fibrillary acidic protein using a second-

- generation microfluidic assay. Validation and comparative analysis with two established assays. *Clin Chem Lab Med* 2024;62:1591–601.
29. Oris C, Khatib-Chahidi C, Pereira B, Bailly Defrance V, Bouvier D, Sapin V. Comparison of GFAP and UCH-L1 measurements using two automated immunoassays (i-STAT® and Alinity®) for the management of patients with mild traumatic brain injury: preliminary results from a French single-center approach. *Int J Mol Sci* 2024;25:4539.
30. Zyliftari S, Luger S, Blums K, Barthelmes S, Humm S, Baum H, et al. GFAP point-of-care measurement for prehospital diagnosis of intracranial hemorrhage in acute coma. *Crit Care* 2024;28:109.
31. Boulo S, Kuhlmann J, Andreasson U, Brix B, Venkataraman I, Herbst V, et al. First amyloid β 1-42 certified reference material for re-calibrating commercial immunoassays. *Alzheimer's Dement* 2020;16:1493–503.
32. Kobeissy F, Arja RD, Munoz JC, Shear DA, Gilsdorf J, Zhu J, et al. The game changer: UCH-L1 and GFAP-based blood test as the first marketed in vitro diagnostic test for mild traumatic brain injury. *Expert Rev Mol Diagn* 2024;24:67–77.
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