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Serum GFAP as a biomarker for progression in multiple sclerosis: assay comparison and a large reference database of healthy controls

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Abstract

Objectives: Compare Elecsys (Roche) and Simoa (Quanterix) immunoassays for serum glial fibrillary acidic protein (GFAP) using our reference database and Z scores, and evaluate their prognostic value for progression independent of relapse activity (PIRA) in multiple sclerosis (MS).

Methods: Platform correlation was assessed in 612 samples from healthy controls (n=188; median [interquartile range, IQR] age 45.1 [36.4–61.7] years) and people with MS (n=424; 45.3 [35.2–53.9] years). Elecsys values were converted to Z scores via Passing-Bablok-derived regression and validated in fingolimod (n=414), and B-cell depleting therapy (BCDT; n=353) cohorts. Z scores and hazard ratios (HRs) for time-to-PIRA were compared using Cox regression.

Results: $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ measurements were correlated (r=0.94), with Elecsys values ~54 % lower ($GFAP_{E-lecsys}$, ng/L=2.847 [95 % confidence interval, CI: 1.335 – 4.98] + 0.457 [0.434 – 0.478] * $GFAP_{Simoa}$, ng/L). In univariable Cox models, $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ Z scores were associated with time-to-PIRA in both validation cohorts. In multivariable Cox models, higher $GFAP_{Simoa}$ Z scores were associated with shorter time-to-PIRA in fingolimod cohort (HR: 1.27 [95 % CI 1.08 – 1.50], p=0.0031) and trended toward significance in BCDT (1.18 [0.99 – 1.41, p=0.0693). In contrast, $GFAP_{Elecsys}$ Z scores were associated with time-to-PIRA in both cohorts (fingolimod: 1.27 [1.09 – 1.48], p=0.0023; BCDT: (1.19 [1.00 – 1.40], p=0.0487).

Conclusions: Serum GFAP measured by Elecsys shows a comparable association with time-to-PIRA as Simoa, and

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$GFAP_{Simoa}$ Z scores can be successfully bridged to $GFAP_{Elecsys}$ Z scores, supporting Elecsys`s potential for clinical implementation.

Keywords: Elecsys assay; serum GFAP; progression independent of relapse activity (PIRA); multiple sclerosis; Simoa assay; Z scores

Introduction

Glial fibrillary acidic protein (GFAP) is an intermediate filament protein expressed by astrocytes. Elevated GFAP levels in cerebrospinal fluid and blood have been associated with disease progression in multiple sclerosis (MS) [1–5].

Conventional enzyme-linked immunosorbent assay (ELISA) can measure GFAP in blood but usually only detects higher concentrations, such as those found in acute neurological injuries for neurological conditions [6, 7]. In less acute conditions such as progressive MS, blood GFAP levels are substantially lower, necessitating the development of ultrasensitive detection methods like the single molecule array (Simoa) [3]. This assay enabled reliable measurement of low-abundance GFAP in blood, leading to a growing body of literature linking elevated GFAP levels to increased risk of progression in MS [4, 8, 9].

Despite its analytical sensitivity, maintaining lot-to-lot consistency with the Simoa assay has been a challenge. The assay uses two antibodies from Banyan Biomarkers (a mouse monoclonal IgG as the capture antibody and a rabbit polyclonal antibody for detection) reported to bind full-length GFAP as well as GFAP breakdown products, likely targeting the central rod domain of the protein [7, 10, 11]. More recently, GFAP has also been introduced as an electrochemiluminescence-based Elecsys assay (Roche Diagnostics AG), which runs on the widely used, automated Cobas platform (Roche Diagnostics AG) and is certified for *in vitro* diagnostic use. The antibodies used in the Elecsys assay are proprietary.

We recently established a reference cohort of over 4,000 healthy controls with serum GFAP concentrations measured by Simoa, confirming that GFAP is strongly associated with age-, sex- and body mass index (BMI), and enabling the calculation of adjusted Z scores or percentiles relative to matched controls [5]. These scores have been shown to strongly predict progression independent of relapse activity (PIRA) in MS patients treated with fingolimod or B-cell depleting therapy (BCDT), using samples collected one year after treatment initiation [4, 5].

In this study, we derived GFAP Z scores from Elecsys measurements and evaluated their ability to predict PIRA in two large MS cohorts treated with fingolimod or BCDT [4, 5]. We conducted a head-to-head comparison of serum GFAP concentrations measured with the Simoa or Elecsys assay, assessing their prognostic performance and the potential of the Elecsys platform as a clinically viable alternative.

Materials and methods

Study design

This observational cohort study aimed to bridge serum GFAP measurements derived from the Simoa ($GFAP_{Simoa}$) and Elecsys ($GFAP_{Elecsys}$) immunoassays and to validate $GFAP_{Elecsys}$ Z scores for predicting PIRA in MS.

We used a 'bridging cohort' of healthy controls (HC) and people with MS (pwMS) from the Swiss MS Cohort (SMSC, NCT02433028) [12], along with two independent validation cohorts from the SMSC treated with fingolimod or BCDT. The analysis used prospectively collected data between 2012 and 2024 (extracted on May 29, 2024) from pwMS enrolled in the SMSC, a multicentric study conducted at eight academic centers in Switzerland. The study protocol has been previously published [13].

Relevant demographic and clinical data were collected, including age, self-reported sex, BMI, and estimated glomerular filtration rate (eGFR). For pwMS, additional variables included Expanded Disability Status Scale (EDSS) score, disease duration, MS subtype, relapse occurrence and current treatment. One serum sample per individual was analysed.

This study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines.

Study population

'Bridging cohort'

To capture a representative concentration range of serum GFAP, the 'bridging cohort' combined samples from HC and pwMS. Specifically, 188 serum samples from HC aged 24 to 75 years were selected from the previously established $GFAP_{Simoa}$ reference database [5, 14–19]. In addition, 424 serum samples from SMSC pwMS receiving a range of disease-modifying therapies (DMTs) were included (33 targeted 'Low to High' samples covering the typical GFAP range, 81 samples during relapse and remission to capture clinically relevant elevations, and 310 routine clinical samples reflecting the real-world distribution) [12].

Validation cohorts

Eligible pwMS who had initiated either fingolimod ('fingolimod') or BCDT ('BCDT') and had completed at least three (half-) yearly clinical visits under the respective treatment, were enrolled at the Basel SMSC center. For each patient, an index serum sample collected under stable treatment conditions was selected eight to 24 months after treatment initiation (median: 12 months). Patients in the validation cohorts had no overlap with those included in the 'bridging cohort'.

After defining the fingolimod and BCDT cohorts, we compared $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ concentrations in the 'bridging cohort' to derive a conversion formula. This formula was then used to calculate $GFAP_{Elecsys}$ Z scores based on the previously established Simoa reference database [19]. Finally, we evaluated and compared the predictive value of both assays (i.e., $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ Z scores) for time-to-PIRA in the validation cohorts.

Clinical measures: disability worsening and PIRA

Disability was assessed with EDSS [20] at each visit by Neurostatus-EDSS certified raters. A relapse was defined as an episode of new, worsening or recurrent neurological disturbance lasting for at least 24 h [21]. PIRA was defined as an EDSS increase using a roving [22] baseline (≥1.5 points from EDSS 0, ≥1.0 point from EDSS 1.0–5, or ≥0.5 point from EDSS ≥5.5), confirmed at a subsequent visit at least 6 months later, with no relapses between the reference and confirmation visit.

GFAP measurements

Blood samples were collected, processed onsite to isolate serum, aliquoted, and stored at −80 °C. Serum GFAP concentrations were measured according to the manufacturer's instructions using the Neuro-2-plex B Simoa assay on the HD-X platform (Quanterix, Billerica, MA) and the Elecsys assay on the Cobas e 402 module, the latter employing two monoclonal anti-GFAP antibodies, one biotinylated and one ruthenium-labelled, in a sandwich immunoassay format. The inter-assay coefficient of variation (CV) for the Simoa assay was 12.0 % [4]. For the Elecsys assay, inter-assay CVs across 11 runs were 5.0 % at a mean concentration of 30 ng/L and 3.9 % at 320 ng/L, based on two internal serum controls. All measurements were done at the University Hospital Basel.

Z scores were calculated as previously described [19], representing the deviation of serum GFAP levels from the healthy reference population in numbers of standard deviations from the mean. These Z scores reflect biomarker abnormality relative to age-, sex- and BMI- matched HC, and can interchangeably also be expressed as percentiles (e.g., a Z score of 1 reflects the 84.1st percentile).

Statistical analyses

Descriptive statistics were reported as counts and percentages for categorial variables, and as medians with interquartile ranges [IQR] for continuous variables. Passing-Bablok regression (R package mcr) and Pearson correlation were used to compare assays.

To assess whether the association between $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ varied by group (HC vs. MS), a linear regression model was fitted with log-transformed $GFAP_{Elecsys}$ as the dependent variable, and log-transformed $GFAP_{Simoa}$, group, and their interaction as predictors. Model estimates were back-transformed and interpreted as percentage changes.

The conversion formula from the Passing-Bablok regression was used to transform $GFAP_{Elecsys}$ to corresponding $GFAP_{Simoa}$ concentrations and GFAP Z scores calculated as described previously [5]. Pathological GFAP Z scores were classified using predefined cut-offs of >0.75 for the fingolimod cohort [5] and >1.0 for the BCDT cohort [4]. To facilitate application, we extended our previously developed online GFAP Z score calculator based on Simoa measurements [5] to incorporate $GFAP_{Elecsys}$ based data (see Results for access details).

The predictive value of continuous GFAP Z scores for time-to-PIRA was assessed using Cox regression. To investigate the effect of confounding factors, multivariable Cox regression models were performed with the additional covariates age, BMI, sex, EDSS, and recent relapse, i.e., within 90 days from sampling.

All analyses were done in R version 4.3.3 and p-value below 0.05 was considered statistically significant.

Results

Study population

The 'bridging cohort' comprised 612 serum samples, including 188 HC (median [IQR] age 45.1 [36.4–61.7] years; 55.9 % female) and 424 pwMS (45.3 [35.2–53.9] years; 67.2 % female; Table 1).

Table 1: Characteristics of the 'bridging cohort'.

	Overall	HC	MS
n	612	188	424
Age, years	45.1 [35.4–55.6]	45.1 [36.4–61.7]	45.3 [35.2–53.9]
Sex=female	390 (63.7)	105 (55.9)	285 (67.2)
BMI, kg/m^2	24.3 [21.8–27.9]	25.4 [22.6–29.4]	24.0 [21.3–27.1]
Disease duration, years	12.8 [6.6–20.5]	N/a	12.8 [6.6–20.5]
Relapse within 90 days=yes	33 (7.7)	N/a	33 (7.7)
EDSS	2.0 [1.5–3.0]	N/a	2.0 [1.5–3.0]
$GFAP_{Simoa}$, ng/L	82.1 [60.1–117.6]	83.5 [58.1–115.5]	81.1 [60.8–117.5]
$GFAP_{Elecsys}$, ng/L	41.5 [30.8–57.5]	44.9 [31.6–60.1]	39.9 [30.1–56.4]

Continuous variables presented as median [IQR], categorial variables presented as count with proportion. BMI, body mass index; EDSS, expanded disability status scale; GFAP, glial fibrillary acidic protein; HC, healthy controls; IQR, interquartile range; MS, multiple sclerosis; n, number; N/a, not applicable.

The validation cohorts included 414 pwMS treated with fingolimod (median [IQR] age 40.3 [31.0–47.8] years; EDSS 2.0 [1.5–3.0]; 65 % female) and 353 pwMS treated with BCDT (age 42.9 [32.9–52.1] years; EDSS 3.0 [2.0–4.5]; 65.7 % female). During follow-up (fingolimod: median [IQR] 9.1 [7.0–11.0] years; BCDT: 4.8 [3.6–5.7] years) PIRA occurred in 31.4 % of fingolimod-treated and 26.9 % BCDT-treated pwMS. Serum samples for both validation cohorts were collected at a similar timepoint, median [IQR] 1.0 [0.9–1.3] year after DMT initiation (Supplementary Table 1 and 2).

Comparisons of $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ measurements

'Bridging cohort'

$GFAP_{Simoa}$ and $GFAP_{Elecsys}$ correlated strongly (r=0.944 [95 % confidence interval, CI: 0.935–0.952], p<0.0001), with 54 % lower values in $GFAP_{Elecsys}$ and a minor systematic bias (i.e., intercept different from 0; Figure 1).

Although the regression slopes differed slightly between HC and pwMS (healthy controls: 0.482 [95 % CI 0.429–0.540]; MS: 0.452 [0.427–0.472], the interaction term was not statistically significant (p=0.1; Supplementary Table 3). This indicates that the association between $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ did not differ meaningfully between the two groups.

The conversion formula was: $GFAP_{Elecsys}$, ng/L=2.847 [95 % CI 1.335 – 4.98] + 0.457 [0.434 – 0.478] * $GFAP_{Simoa}$, ng/L.

Validation cohorts

In both the cohorts, serum $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ showed strong correlations: r=0.876 [95 % CI 0.851–0.897] in fingolimod and r=0.892 [0.868–0.911] in BCDT (both p<0.0001; Supplementary Figure 1A – B).

In both cohorts, $GFAP_{Elecsys}$ concentrations were consistently lower than $GFAP_{Simoa}$ concentrations (approximately 52 % in fingolimod and 50 % lower in BCDT). In the

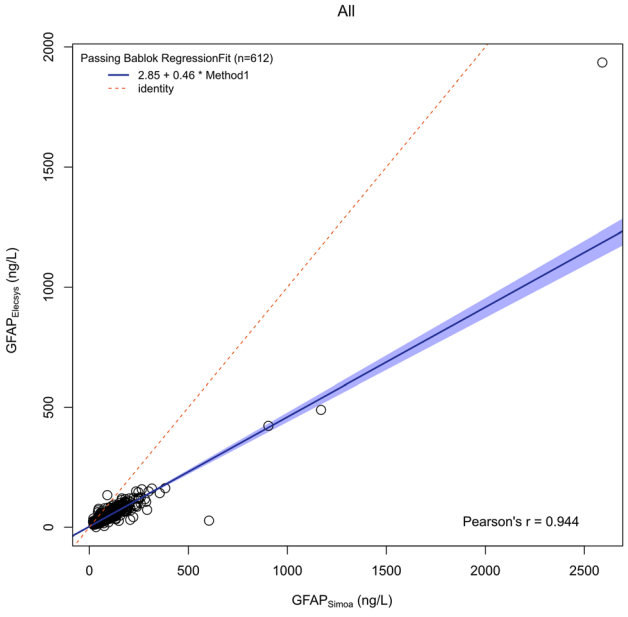

Figure 1: Method comparison of $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ by Passing-Bablok regression analysis in the 'bridging cohort' consisting of healthy controls and pwMS. Scatter plot shows individual serum GFAP concentrations (n=612) measured using Simoa (x-axis) and Elecsys (y-axis). The solid black line represents the Passing-Bablok regression fit, and the shaded area indicates the 95 % CI. The dashed line represents the line of identity (y=x), representing perfect agreement between the two assays. The Pearson correlation coefficient (r=0.944) indicates a strong linear relationship between the measurements of the two platforms. The regression equation was: $GFAP_{Elecsys}$=2.847 [95 % CI: 1.288–4.636] + 0.457 [0.435–0.477] * $GFAP_{Simoa}$. CI, confidence interval; GFAP, glial fibrillary acidic protein; pwMS, people with multiple sclerosis.

BCDT cohort, a proportional bias (but not systematic bias) between assays was observed (Supplementary Figure 1B).

Based on the conversion formula from the 'bridging cohort', $GFAP_{Elecsys}$ concentrations were converted to $GFAP_{Simoa}$. The resulting GFAP Z scores correlated well between methods: r=0.827 [95 % CI 0.794–0.855] in fingolimod (Figure 2A) and r=0.834 [0.800–0.863] in BCDT (Figure 2B).

To support clinical and research applications, we have extended our previously developed online GFAP Z score calculator [5], originally based on Simoa measurements, to also include $GFAP_{Elecsys}$. The updated tool is accessible at https://shiny.dkfbasel.ch/baselgfapreference/.

When classifying pathological GFAP Z scores using predefined cut-offs, the agreement between the assays was high: 87.4 % agreement in fingolimod and 88.1 % in BCDT. 12.6% and 11.9 %, respectively, were discordantly classified, with Z scores exceeding the cut-off in one assay but not the other.

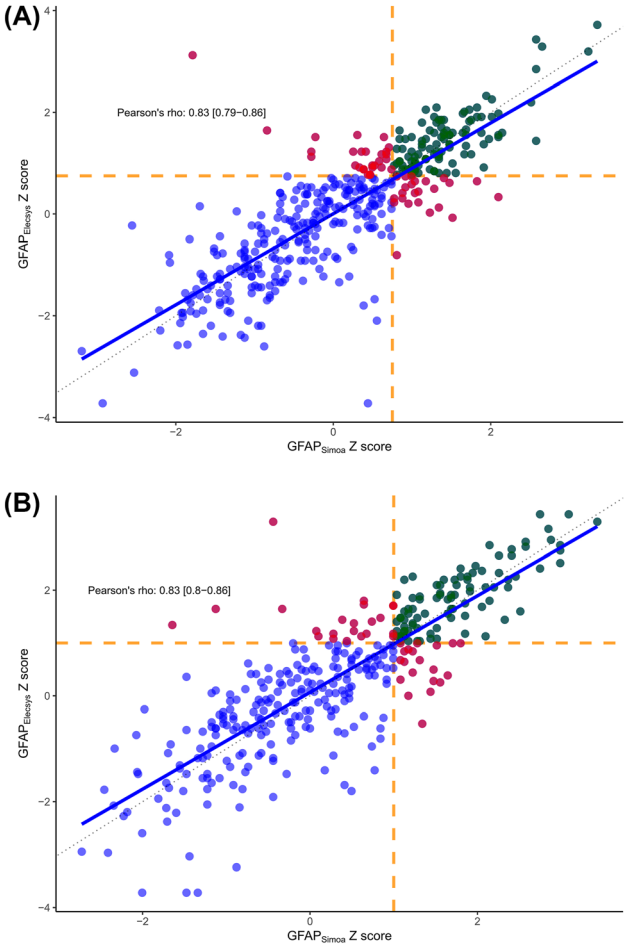

Figure 2: Correlations between $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ Z scores in fingolimod and BCDT cohorts. Correlation and agreement between $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ Z scores in (A) the fingolimod (n=414) and (B) BCDT (n=353) cohorts. Pearson's correlation coefficients were 0.827 [95 % CI: 0.794–0.855] and 0.834 [0.800–0.863], respectively. Agreement in classification of pathological vs. non-pathological Z scores was 87.4 % (Z score >0.75) in (A) and 88.1 % (Z score >1) in (B). Green points: classified as pathological by both assays; blue points: classified as non-pathological by both assays; and red points: discordant classifications between assays. Orange dotted lines mark Z score thresholds; the grey dotted line represents the line of equality (x=y), indicating perfect agreement between assays; the solid blue line shows the Passing-Bablok regression of $GFAP_{Elecsys}$ Z scores on $GFAP_{Simoa}$ Z scores. BCDT, B-cell depleting therapy; CI, confidence interval; GFAP, glial fibrillary acidic protein.

Prediction of PIRA by $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ Z scores in the validation cohorts

In both cohorts, each unit increase in $GFAP_{Simoa}$ Z score was associated with a significantly increased risk of PIRA in univariable Cox regression models: a 24 % in fingolimod (hazard ratio [HR]: 1.24 [95 % CI 1.06–1.46], p=0.0066) and 25 % in BCDT (1.25 [1.06–1.48], p=0.0080). Similarly, $GFAP_{Elecsys}$ Z score showed nearly identical associations, with 25 % increase in fingolimod (HR 1.25 [95 % CI 1.08–1.45], p=0.0033) and 26 % in BCDT (1.26 [1.07–1.47], p=0.0044), Table 2.

These associations remained in multivariable models adjusting for age, BMI, sex, EDSS, and recent relapse, with GFAP Z scores (both assays) conferring a 27 % increased risk of PIRA per unit Z score increase in fingolimod ($GFAP_{Simoa}$: HR: 1.27 [95 % CI 1.08–1.50], p=0.0031; $GFAP_{Elecsys}$: 1.27 [1.09–1.48], p=0.0023). In BCDT, adjusted HRs showed an 18 % increase for $GFAP_{Simoa}$ (1.18 [0.99–1.41], p=0.0693) and 19 % for $GFAP_{Elecsys}$ (1.19 [1.00–1.40], p=0.0487) per Z score unit increase (Table 2).

Discussion

GFAP is a promising biomarker for MS progression [8, 23, 24], but clinically scalable measurement methods remain limited to date. In this study, we show that serum GFAP concentrations measured using the $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ assays are strongly concordant and offer comparable prognostic value for PIRA in MS. To enable standardization of $GFAP_{Elecsys}$ measurements, we derived $GFAP_{Elecsys}$-based Z scores using a Passing-Bablok regression calibrated against our previously defined healthy reference cohort, which was originally based on $GFAP_{Simoa}$ measurements [5]. We validated the $GFAP_{Elecsys}$ Z scores in two independent MS cohorts treated with fingolimod or BCDT. Cox regression analyses confirmed that GFAP Z scores derived from both assays were similarly associated with future PIRA. These findings support the potential of the $GFAP_{Elecsys}$ assay for broader clinical implementation.

Table 2: Cox models for the association of $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ Z scores with time-to-PIRA in the fingolimod and BCDT cohorts.

	Fingolimod cohort (n=414)				BCDT cohort (n=353)			
	$GFAP_{Simoa}$		$GFAP_{Elecsys}$		$GFAP_{Simoa}$		$GFAP_{Elecsys}$	
	HR [95 % CI]	p-Value	HR [95 % CI]	p-Value	HR [95 % CI]	p-Value	HR [95 % CI]	p-Value
Univariable model								
GFAP Z score (per unit increase)	1.24 [1.06–1.46]	0.0066	1.25 [1.08–1.45]	0.0033	1.25 [1.06–1.48]	0.0080	1.26 [1.07–1.47]	0.0044
Multivariable model								
GFAP Z score (per unit increase)	1.27 [1.08–1.50]	0.0031	1.27 [1.09–1.48]	0.0023	1.18 [0.99–1.41]	0.0693	1.19 [1.00–1.40]	0.0487
Age, year (per year increase)	1.02 [1.00–1.04]	0.0126	1.02 [1.00–1.04]	0.0175	1.02 [1.00–1.04]	0.0236	1.02 [1.00–1.04]	0.0223
BMI, kg/m^2 (per unit increase)	1.02 [0.99–1.06]	0.1855	1.02 [0.99–1.06]	0.2145	1.00 [0.96–1.04]	0.9579	1.00 [0.96–1.04]	0.9436
Sex, female vs. male	0.86 [0.60–1.23]	0.4155	0.85 [0.59–1.21]	0.3604	0.71 [0.47–1.07]	0.1049	0.67 [0.44–1.03]	0.0688
EDSS	0.95 [0.82–1.09]	0.4475	0.94 [0.81–1.08]	0.3807	1.29 [1.13–1.46]	0.0001	1.28 [1.12–1.45]	0.0002
Recent relapse (<90 days)	1.00 [0.43–2.32]	0.9951	0.98 [0.42–2.27]	0.9623	0.92 [0.22–3.79]	0.9066	0.92 [0.22–3.81]	0.9095

Estimates represent HRs, i.e., an estimate of 1.24 means a 24 % higher risk of PIRA with every Z score unit increase of GFAP. BCDT, B-cell depleting therapy; BMI, body mass index; CI, confidence interval; EDSS, expanded disability status scale; HR, hazard ratio; GFAP, glial fibrillary acidic protein; PIRA, progression independent of relapse activity.

Building on our main findings, the strong linear correlation of $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ measurements, demonstrated both in this study and in prior work [25], along with consistent proportional bias (~54 %) support the use of a conversion formula to leverage our existing GFAP reference database [5]. Unlike absolute GFAP concentrations, Z scores reflect the degree of biomarker abnormality relative to a normative population, accounting for physiological influences such as age, sex and BMI [5]. The high correlation of Z scores derived from both assays in the validation cohorts (87–88 %) underscores the robustness of this conversion approach.

Although our findings demonstrate strong concordance and comparable prognostic value between $GFAP_{Simoa}$ and $GFAP_{Elecsys}$, the assays differ in analytical performance and clinical feasibility. Despite its clinical utility, the $GFAP_{Simoa}$ assay`s implementation may be limited by higher analytical variability, sometimes exceeding the recommended 20 % CV [26]. In contrast, the $GFAP_{Elecsys}$ assay has demonstrated improved reproducibility and strong correlations with the $GFAP_{Simoa}$ results [25, 27]. Importantly, it`s integration with standard clinical chemistry platforms (e.g., Roche Cobas modules) positions $GFAP_{Elecsys}$ as a feasible tool for reliable longitudinal use in clinical settings.

The study has limitations. First, not all samples across cohorts were measured in parallel, and minor day-to-day variability (e.g., room temperature) may have differently affected the assays. However, such pre-analytical effects have not been reported in systematic studies [28, 29]. Second, the $GFAP_{Elecsys}$ assay uses proprietary antibodies, and the GFAP epitope targeted is unknown. As GFAP exists in multiple isoforms and undergoes post-translational modifications and cleavage, potentially in a disease-specific manner, epitope variability may influence assay performance [11]. Nevertheless, both assays showed consistent associations with demographic variables (age, sex, BMI) and MS-related features (recent relapse, EDSS, and PIRA), suggesting no systematic bias linked to these factors. Moreover, the strong correlation and comparable prognostic performance observed in clinical validation support the robustness of both assays. Third, we could not assess lot-to-lot variability for $GFAP_{Elecsys}$ because all measurements were generated from a single reagent lot. Nonetheless, Elecsys assays typically show lower lot-to-lot variability than research-use-only platforms such as Simoa.

In conclusion, our results demonstrate that Z scores from the $GFAP_{Simoa}$ assay can be reliably translated to corresponding $GFAP_{Elecsys}$ Z scores. We further show that serum GFAP measured using the $GFAP_{Simoa}$ and $GFAP_{Elecsys}$ assays show strong concordance and comparable predictive value for future PIRA in MS. Given its analytical robustness and compatibility with existing clinical chemistry platforms, our findings support the adoption of the $GFAP_{Elecsys}$ assay for monitoring progression risk in MS patients. To support clinical implementation, we have extended our previously developed online GFAP Z score calculator to include $GFAP_{Elecsys}$-based values (https://shiny.dkfbasel.ch/baselgfapreference/).

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Research ethics: This cohort study, conducted since January 1, 2012, followed the Declaration of Helsinki and was approved by the Ethics Committees of all participating centers.

Informed consent: Written informed consent was obtained from all HCs and pwMS.

Author contributions: Significant contribution to: conception and design of the study (E.A.J.W., S.Sa., P.B., S.Sc., A.M.M., J.O., N.G., D.L., M.E., and J.K.), acquisition and analysis of data (E.A.J.W., S.Sa., P.B., S.Sc., A.M.M, J.O., N.G., K.B., M.Herm., S.M., S.F., J.F.V.G., A.Z., G.D., M.D., C.G., C.P.K., C.P., C.Z., P.H.L., R.H., M.Herw., C.G., D.L., M.E. and J.K.), participation in drafting a significant portion of the manuscript or figures (E.A.J.W., S.Sa., P.B., S.Sc., and J.K.). All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Data availability: Written requests for access to the data reported in this paper will be considered by the corresponding author and a decision made about the appropriateness of the use of the data. If the use is appropriate, a data sharing agreement will be put in place before a fully de-identified version of the dataset used for the analysis with individual participant data is made available. The internet-

based application for determination of sGFAP Z scores is available at: https://shiny.dkfbasel.ch/baselgfapreference/.

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