Short-term biological variation of serum glial fibrillary acidic protein

Christensen, Silje Hovden; Hviid, Claus Vinter Bødker; Madsen, Anne Tranberg; Parkner, Tina; Winther-Larsen, Anne

Published in:
Clinical Chemistry and Laboratory Medicine

DOI (link to publication from Publisher):
10.1515/cclm-2022-0480

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

Silje Hovden Christensen, Claus Vinter Bødker Hviid, Anne Tranberg Madsen, Tina Parkner and Anne Winther-Larsen*

Short-term biological variation of serum glial fibrillary acidic protein

https://doi.org/10.1515/cclm-2022-0480
Received May 17, 2022; accepted July 29, 2022;
published online August 15, 2022

Abstract

Objectives: Serum glial fibrillary acidic protein (GFAP) is an emerging biomarker for intracerebral diseases and is approved for clinical use in traumatic brain injury. GFAP is also being investigated for several other applications, where the GFAP changes are not always outstanding. It is thus essential for the interpretation of GFAP to distinguish clinical relevant changes from natural occurring biological variation. This study aimed at estimating the biological variation of serum GFAP.

Methods: Apparently healthy subjects (n=33) had blood sampled for three consecutive days. On the second day, blood was also drawn every third hour from 9 AM to 9 PM. Serum GFAP was measured by Single Molecule Array (Simoa™). Components of biological variation were estimated in a linear mixed-effects model.

Results: The overall median GFAP value was 92.5 pg/mL (range 34.4–260.3 pg/mL). The overall within– (CV_I) and between-subject variations (CV_G) were 9.7 and 39.5%. The reference change value was 36.9% for an increase. No day-to-day variation was observed, however semidiurnal variation was observed with increasing GFAP values between 9 AM and 12 PM ($p<0.00001$) and decreasing from 12 to 9 PM ($p<0.001$).

Conclusions: Serum GFAP exhibits a relatively low CV_I but a considerable CV_G and a marked semidiurnal variation. This implies caution on the timing of blood sampling and when interpreting GFAP in relation to reference intervals, especially in conditions where only small GFAP differences are observed.

Keywords: biological variation; biomarkers; depressive disorder; glial fibrillary acidic protein; individual; nervous system diseases.

Introduction

Glial acidic fibrillary protein (GFAP) is an astrocyte cytoskeletal protein involved in maintenance of the blood-brain-barrier and intercellular connections [1]. The protein is considered a signature of astrocytes [2, 3] and is released following astrocyte damage or activation making it an promising biomarker of intracerebral injury and diseases [1, 4]. However, the utility as a blood biomarker was challenged by the previously limited sensitivity of available assays [3] and profound increases or breakage of the blood-brain-barrier were required for assessment of GFAP in blood [1, 3, 5]. Yet, with the development of ultra-sensitive assays, such as the single molecule array (Simoa), GFAP can now be measured at picomolar levels in blood, enabling broad investigations of its biomarker potential.

Subsequently, blood levels of GFAP has been extensively investigated in mild traumatic brain injury and are applied in clinical practice [1, 3]. Furthermore, GFAP also shows potential as a biomarker in intracerebral vascular events [1, 3], neuro-inflammatory and neurodegenerative disorders [3, 6], and in patients with depression where initial studies have found increased GFAP levels in cerebrospinal fluid [7] and serum [8].

While evidence for the biomarker potential of blood GFAP measurements is growing, basic aspects of GFAP regulation, pre-analytical and analytical issues remains to be clarified [3]. In this context, the biological variation of GFAP has not been investigated previously. Yet, multiple

***Corresponding author: Anne Winther-Larsen**, MD, PhD, Associated Professor, Department of Clinical Biochemistry, Aarhus University Hospital, Palle Juul-Jensens Boulevard 99, 8200, Aarhus N, Aarhus, Denmark; and Department of Clinical Medicine, Aarhus University, Aarhus, Denmark, Phone: +0045 7844 0000, E-mail: anlarsen@rm.dk
Silje Hovden Christensen, Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark
Claus Vinter Bødker Hviid, Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; Department of Clinical Biochemistry, Aalborg University Hospital, Aarhus, Denmark; and Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
Anne Tranberg Madsen, Department of Microbiology and Immunology, Albert Einstein College of Medicine, New York, NY, USA. https://orcid.org/0000-0001-9146-5943
Tina Parkner, Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark; and Department of Clinical Medicine, Aarhus University, Aarhus, Denmark

studies report age-dependent increases in GFAP, which indicates a considerable between-subject variation [3, 9–11]. Especially in conditions where only subtle changes in GFAP-levels are detected, the knowledge of biological variation is important to consider when evaluating if an observed change in GFAP-level is actually owing to changes in disease or caused by random variation.

The current study aimed to establish estimates of the biological variation of serum GFAP in healthy individuals, and to define its reference change value (RCV) and the index of individuality (II).

Materials and methods

Subjects

Apparently healthy subjects were enrolled between June and October 2018 at Aarhus University Hospital, Denmark. A detailed presentation of the study population including in- and exclusion criteria has previously been described [12]. Briefly, subjects were included if they were >18 years old and none of the following conditions were present: pregnant, smoking, receiving medical treatment, suffering from chronic infections or inflammations. Furthermore, acute infections, night-shift work or crossing of time-zones in the week before sampling lead to exclusion.

The study was approved by the Central Denmark Region Committees on Biochemical Research Ethics (1-10-72-452-17) and was conducted in accordance with the Helsinki Declaration. All subjects provided written confirmed consent.

Study design

The study was designed in accordance with the checklists for biological variation studies [13–15].

Blood were sampled by venepuncture at 9 AM in three consecutive days. On the second day blood was also drawn over a 12 h period at the following time points: 12, 3, 6, and 9 PM. All blood draws were performed by a small team of laboratory technicians. Subjects refrained from food and physical activity 1 h before blood sampling, and from alcohol as well as hard physical activity (walking and low intensity bicycling not included) during the duration of the study.

Laboratory analysis

The sample handling has been described previously [12, 16]. At each time-point blood was collected in 10 mL serum tubes (BD vacutainer®) and left to incubate for 30 min before centrifugation for 10 min at 1,800 g at room temperature (22–24 °C). Finally, they were frozen at −80 °C until analysis.

GFAP quantification was performed on the Simoa™ HD-1 platform (Quanterix©, Lexington, MA, USA), which applies single-molecule enzyme-linked immunosorbent assays (digital ELISA) in detection of molecules at ultrasensitive levels [17]. The GFAP Discovery kit (Quanterix©, Lexington, MA, USA) was used in accordance with manufacturer's instructions including a 4-fold dilution of the samples. According to the manufacturer, the GFAP Discovery kit has a dynamic range from 0–4,000 pg/mL (standard curve 0.211–1,000 pg/mL) and limits of detection and quantification of 0.211 and 0.686 pg/mL, respectively.

The GFAP discovery kit analytical performance was tested prior to the study applying internal quality controls fabricated in the laboratory with serum from random patients spiked with serum from patients with known elevated levels of GFAP (subarachnoid haemorrhage). All samples in the present study were analysed on half plates, with the internal quality controls located in the beginning and end of the plate. The inter-serial precision was 18.4% (at a level of 154.0 pg/mL) and 16.5% (at a level of 1936.4 pg/mL).

All samples including controls were analysed in duplicates by the same laboratory technician. All samples from each individual were batch analysed. GFAP Discovery kits from the same lot were used for all runs.

Statistical analysis

The data distribution of each subject was assessed by visual evaluation and by the Shapiro-Wilk test. As data followed a ln-normal distribution, it was ln-transformed, and the results were presented as median and range. A linear regression of the median value for each blood drawing vs. the blood drawing number was performed to evaluate the steady state of the subjects. Variance homogeneity of within-subject variability was assessed by Brown-Forsythe test on the ln-transformed data. Potential outliers were investigated using the Cochran's C test for determining analytical and within-subject outliers, and the Dixon-Reed method for determining between subject outliers [15, 18].

The analytical variation (CV_A) was estimated according to Fraser et al. from duplicates of every sample [18], whereas the within-subject variation (CV_I) and between-subject variation (CV_G) were estimated using linear mixed effects models with day, sample, and age as fixed effects and subjects as a random effect. Age was included as a fixed effect after using Pearson's correlation on ln-transformed data to investigate the correlation of GFAP and age, because studies indicate increasing GFAP with increasing age [9, 10]. For all the biological variation estimates, 95% confidence intervals (CIs) were calculated according to Roraas et al. [19].

Index of individuality (II) and number of samples required to estimate an individual's homeostatic set-point (n) within ± 15% with 95% confidence were calculated with the equations proposed by Fraser et al. [18]: $II=\sqrt{(CV_I^2 + CV_A^2)}/CV_G$ and $n=(z \cdot \sqrt{(CVI2 + CV_A^2)}/D)^2$, where z is the z-score and D is the desired percentage closeness to the homeostatic set point. The reference change value (RCV) was calculated on ln-transformed data as $RCV=\exp(\pm z \cdot \sqrt{2} \cdot \sigma) - 1$, where $\sigma=\sqrt{\ln (CV_{I+A}^2 + 1)}$ [20].

For the comparisons of day-to-day and semidiurnal median values, the Bonferroni correction was used to correct for multiple comparisons. $p<0.05$ was considered significant.

Statistical calculations were performed in STATA 14 (StataCorp) and GraphPad Prism 9.3.1 (GraphPad Software).

Results

Subjects

A total of 33 apparently healthy subjects (70% women) with a median age of 39 years (range 22–66) were enrolled. Due to variable participation at different time-points (14 subjects had blood drawn on all seven time-points (7 samples), 14 subjects had all blood draws except from the 6 to 9 PM blood draws (5 samples), one participant had all samples except for the 3, 6, and 9 PM blood draws (4 samples), and four subjects participated only in the morning blood drawings (3 samples)), 184 blood samples were collected. Even though, no outliers were statistically detected on any of the three levels investigated, one subject was excluded from further analysis as this subject had a mean value of GFAP well above the expected in healthy subjects (>300 pg/mL). Exclusion of this subject did not influence the variation estimates except for a minor decrease in CV_G (data not shown). Furthermore, for three samples no GFAP-result was obtained due to analytical error, resulting in a total of 174 samples. All individuals were in steady state, and no heterogeneity of variance was detected.

Indices of variation

The overall median value of GFAP was 92.5 pg/mL (range 34.4–260.3) (Table 1). The individual median and range of GFAP values for every subject are depicted in Figure 1.

Table 1: Components of biological variation.

	GFAP
Number of subjects	32
Number of samples[a]	174
Median, pg/mL	92.5
Range, pg/mL	34.4–260.3
CV_I, %	9.7 (7.6–11.8)
CV_G, %	39.5 (31.7–47.3)
CV_A, %	5.8 (5.1–6.5)
II[b]	0.29
RCV[c], %	
Increasing	36.9
Decreasing	26.9
Samples required, n[d]	>2

Values in parentheses are the 95% confidence intervals. [a]Number of samples analyzed after exclusion of outliers; [b]II, index of individuality; [c]RCV, reference change value at 95% significance; [d]n, required to estimate homeostatic set point within 15% with 95% confidence. CV_I, within-subject coefficient of variation; CV_G, between-subject coefficient of variation; CV_A, analytical coefficient of variation.

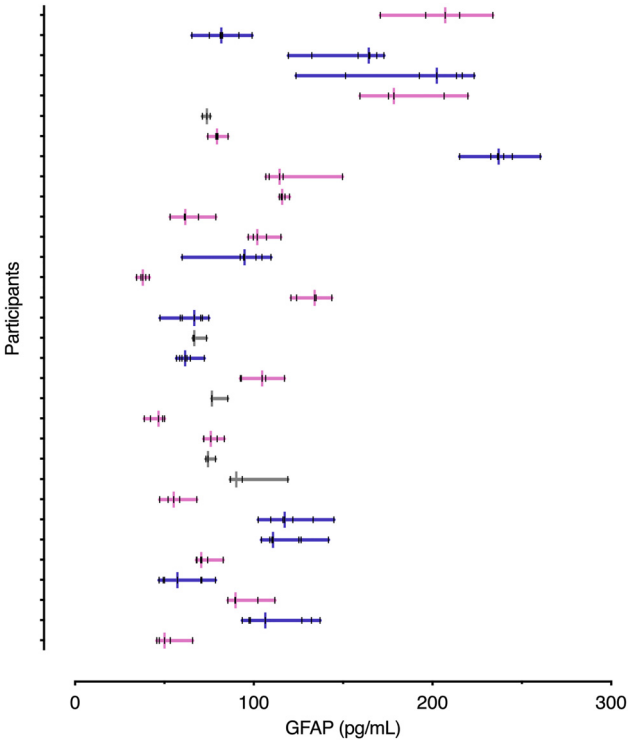

Figure 1: Glial fibrillary acidic protein (GFAP) levels in apparently healthy subjects ordered according to age with the youngest at the bottom (22 years) and the oldest at the top (66 years). Subjects are coloured according to the number of samples contributed by the individual: blue, 7 samples; pink, 5–6 samples; grey, 3–4 samples. The coloured vertical lines indicate the median values and the horizontal lines shows the ranges. The black vertical lines illustrate the mean value of duplicates for each measurement.

A significant increase in GFAP level with increasing age was seen (r=0.50, p<0.0001) (Supplementary Figure S1). The overall CV_I was 9.7% (95% CI 7.6–11.8) and the CV_G was markedly higher at 39.5% (95% CI 31.7–47.3) (Table 1). Accordantly, the II was only 0.29. CV_A was 5.8 (95% CI 5.1–6.5) and, thus, lower than the CV_I. For an increase in serum GFAP, the RCV was 36.9% at a 95% significance level. More than two samples were found to be required to estimate the homeostatic set-point ±15% with 95% confidence.

If age was not considered a fixed effect in the mixed model, a systematic effect of age was observed with increase in CV_G to 45.1% (95% CI 36.7–55.6). Sex had no effect on the CV_G (data not shown).

Day-to-day and semidiurnal ultradian variation

Measurements from the 9 AM blood samples on three consecutive days were used to estimate day-to-day

variance, whereas the semidiurnal variance was calculated from all the five measurements on day two (9 AM, 12, 3, 6, 9 PM). Median values and variance components are shown in Table 2. Regarding day-to-day variance, no systematic difference was observed in GFAP levels between the three days (p=0.6, Figure 2). For semidiurnal variation a statistical significant increase between GFAP values at 9 AM and 12 PM (p<0.00001) was observed, and statistical significant decreases from 12 to 9 PM (p<0.001), as well as from 12 to 3 PM (p<0.05) and 6–9 PM (p<0.005) were observed (Figure 3).

The CV_I of the day-to-day variation was smaller than the overall CV_I and less than half of the CV_I of the semidiurnal variation (5.6% (95% CI 2.9–8.4) vs. 9.7% (95% CI 7.6–11.8) and 11.42 (95% CI 8.8–14.1), respectively). In contrast, the CV_G values were similar (Tables 1 and 2).

Discussion

In this study, we evaluated the biological variation of GFAP and found a relatively low within-subject variation and a moderate RCV. In contrast, the observed between-subject variation was considerable, resulting in a low II. Furthermore, we found that GFAP exhibited semidiurnal variation, whereas no day-to-day variation was observed.

The relatively low overall within-subject variation observed (CV_I 9.7%) implicates that the fluctuations of GFAP around a homeostatic set point in an individual varies only modestly. This is comparable to other brain-derived biomarkers such as the glial protein S100B (CV_I 10.2%) and the neuron-specific enolase (NSE) (CV_I 10.9%) [21], however, higher than the CV_I found for neurofilament light chain (NfL) (CV_I 3.1%) [16]. Thus, brain-derived proteins seem to be rather tightly regulated.

In contrast, the between-subject variation (CV_G 39.5%) was substantially larger, which indicates that the homeostatic set points vary among individuals. Consequently, we reported a low II of only 0.29, reflecting a marked individuality of GFAP. As the II reflects the relationship between the within-subject and between-subject variation, a low II indicates that conventional reference values are of limited utility, because an individual's normal GFAP-values

Table 2: Day-to-day and semidiurnal components of biological variation.

	GFAP
Day-to-day	
Number of subjects	32
Day 1 pg/mL	85.2
Day 2 pg/mL	85.5
Day 3 pg/mL	90.7
CV_I, %	5.6 (2.9–8.4)
CV_G, %	39.5 (31.6–47.4)
Semidiurnal	
Number of subjects	29
12 PM, pg/mL	106.4
03 PM, pg/mL	101.2
06 PM, pg/mL	104.6
09 PM, pg/mL	109.0
CV_I, %	11.4 (8.8–14.1)
CV_G, %	38.9 (31.4–46.5)

Day-to-day and semidiurnal cfDNA levels are presented as medians. Values in parentheses are the 95% confidence intervals. CV_I, within-subject coefficient of variation; CV_G, between-subject coefficient of variation.

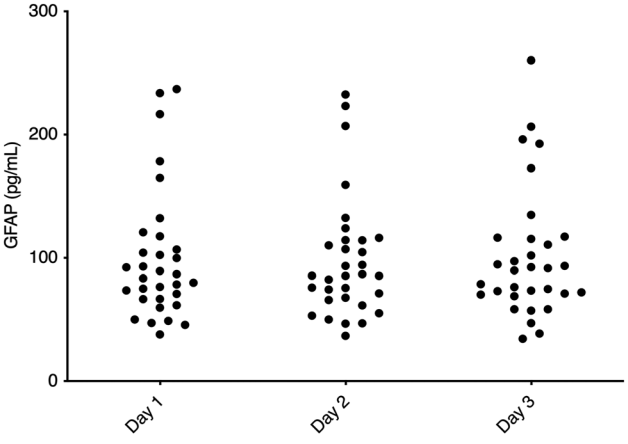

Figure 2: Day-to-day levels of glial fibrillary acidic protein (GFAP). Individual results (spots) are depicted.

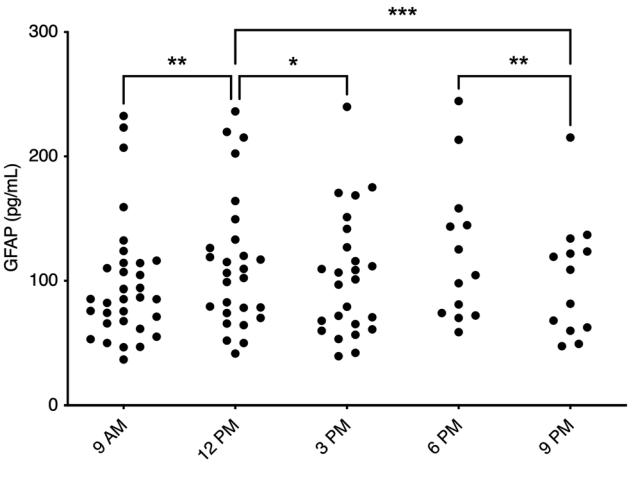

Figure 3: Within-day levels of glial fibrillary acidic protein (GFAP) from the second day.
Individual results (spots) are depicted. Significant differences are marked with *p<0.05, **p<0.005, ***p<0.0005.

(around the homeostatic set point) will only span a small proportion of the reference interval and changes that are abnormal for this individual will not necessarily result in values outside the reference range [15]. An II >1.4 indicates great applicability of conventional reference intervals, whereas an II<0.6 indicates limited applicability [15, 18]. However, reference intervals divided into relevant subgroups (stratified on age, sex, ethnicity, etc.) may still be useful [18]. We did find an even higher between-subject variation if age was not considered a fixed factor, indicating that partitioning on age would be of importance. Conversely, we did not find an effect of sex. Even though only ten men were included in the study, this has been shown to be sufficient to conclude upon according to Braga et al. [15]. Moreover, the finding is in agreement with previous reports of no difference on sex [9]. Previously, we have shown a similarly low II for NfL [16] and have established an age-divided reference interval [22], and this approach may also be useable for GFAP. Nevertheless, instead of using reference intervals, another approach could be to perform repeated measurements of GFAP to evaluate the homeostatic set point of an individual before concluding on the GFAP level. We found that more than two measurements are required to evaluate the homeostatic set point of GFAP.

When monitoring GFAP-levels with serial measurements, as for example proposed in cerebral insults [23], the RCV is important to consider. The RCV is the combination of the analytical and within-subject variation, and provides an estimate on the minimum change required between two serial measurements before a significant difference is observed [18]. For GFAP, the RCV was higher, though comparable, to other brain-derived proteins such as NfL (24.3%) [16], NSE (30.2%), and S100B (29.3%) [21]. A reduction of the RCV would require a better analytical performance of the GFAP assay, where a CV_A of half the CV_I is desirable [18]. In conditions where a blatant rise in GFAP is observed, such as intracerebral haemorrhage [3, 23], the II and RCV is of less importance; however, in disorders such as multiple sclerosis or depression where more discrete changes are observed [8, 9], it has to be taken into consideration.

We found a semidiurnal variation with increasing GFAP values until midday and thereafter decreasing levels. This is in accordance with a previous study indicating a diurnal variation with decreasing plasma GFAP levels during the night (–12.1%) [24]. The findings may reflect a circadian rhythm in the glycophatic drainage of GFAP from the brain. Research indicates that the clearance of solutes from the brain is dependent on arousal state [25], and a circadian rhythm in cerebrospinal fluid has been observed for amyloid-β and tau proteins [26, 27] and amyloid-β in blood [28]. Alternatively, the observed diurnal variation of GFAP may reflect a diurnal rhythm of astrocyte activity. GFAP is up-regulated in activated astrocytes [4, 29], and there is evidence of astrocytes exhibiting a circadian clock function e.g. with changes in glutamate metabolism [30]. No matter the reason for the semidiurnal variation, the timing of blood sampling needs to be taken into consideration in future studies, especially, in conditions where only a small difference is expected.

The strengths of this study are the adherence to the biological variation check-list [13–15] and the application of a strict pre-analytical protocol to diminish the influence of pre-analytical variation on the results. Furthermore, the variation of the analytical phase was also minimized by batch analysis performed by the same technician under a standardised protocol. A limitation of the study was that fewer subjects participated in the 6 and 9 PM blood draws, making these estimates less certain. The relatively short time period investigated may also be considered a limitation, as it renders the monthly or seasonal biological variation unknown. Studies involving less than one week may potentially underestimate the within-subject variation, but often the within-subject variation is considered constant [18]. In estimation of the overall biological variation, components of semidiurnal – and day-to-day variation were pooled. As such, the biological variation may have been overestimated by the potential circadian changes observed. However, the subjects were in steady state, and the current clinical application of GFAP is in emergency settings where repeated sampling is performed within a short timeframe [1, 3, 23]. As such, the inclusion of both variation components in the biological variation estimate reflects the clinical application more accurately than day-to-day variation alone. Another limitation is the fact that the samples had been stored (frozen) for three years and the missing knowledge on long-term stability of GFAP. However, all samples were treated equally. Finally, only healthy individuals were included in the study, and thereby no conclusions on the biological variation of GFAP in diseased individuals can be made. However, it is important to be familiar with the variation in healthy individuals as absence of the expected variation could be a sign of disease [18].

Conclusions

Serum GFAP exhibits a relatively low within-subject variation with no day-to-day variation, but marked semidiurnal

variation. Furthermore, GFAP displays a considerable between-subject variation. Altogether, this warrants caution when interpreting GFAP-levels in conditions where only subtle changes are expected and may limit the utility of ordinary reference intervals in such conditions.

Acknowledgments: The authors wish to thank the biomedical laboratory technicians at the research department of the Department of Clinical Biochemistry, Aarhus University Hospital.
Research funding: The work was supported by Harboefonden and Dagmar Marshalls Fond. The funders did not have any role in the study design, data collection, data analysis, interpretation, or writing of the report.
Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.
Competing interests: Authors state no conflicts of interest.
Informed consent: Informed consent was obtained from all individuals included in this study.
Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Central Denmark Region Committees on Biomedical Research Ethics (1-10-72-452-17).

References

1. Yang Z, Wang KK. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci 2015;38:364–74.
2. Messing A, Brenner M. GFAP at 50. ASN Neuro 2020;12: 1759091420949680. https://doi.org/10.1177/1759091420949680.
3. Abdelhak A, Foschi M, Abu-Rumeileh S, Yue JK, D'Anna L, Huss A, et al. Blood GFAP as an emerging biomarker in brain and spinal cord disorders. Nat Rev Neurol 2022;18:158–72.
4. Kim R, Healey KL, Sepulveda-Orengo MT, Reissner KJ. Astroglial correlates of neuropsychiatric disease: from astrocytopathy to astrogliosis. Prog Neuro-Psychopharmacol Biol Psychiatry 2018; 87:126–46.
5. Janigro D, Mondello S, Posti JP, Unden J. GFAP and S100B: what you always wanted to know and never dared to ask. Front Neurol 2022;13:835597.
6. Benedet AL, Mila-Aloma 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.
7. Michel M, Fiebich BL, Kuzior H, Meixensberger S, Berger B, Maier S, et al. Increased GFAP concentrations in the cerebrospinal fluid of patients with unipolar depression. Transl Psychiatry 2021;11:308.
8. Steinacker P, Al Shweiki MR, Oeckl P, Graf H, Ludolph AC, Schonfeldt-Lecuona C, et al. Glial fibrillary acidic protein as blood biomarker for differential diagnosis and severity of major depressive disorder. J Psychiatr Res 2021;144:54–8.
9. Abdelhak A, Huss A, Kassubek J, Tumani H, Otto M. Serum GFAP as a biomarker for disease severity in multiple sclerosis. Sci Rep 2018;8:14798.
10. Huebschmann NA, Luoto TM, Karr JE, Berghem K, Blennow K, Zetterberg H, et al. Comparing glial fibrillary acidic protein (GFAP) in serum and plasma following mild traumatic brain injury in older adults. Front Neurol 2020;11:1054.
11. Deiner S, Baxter MG, Mincer JS, Sano M, Hall J, Mohammed I, et al. Human plasma biomarker responses to inhalational general anaesthesia without surgery. Br J Anaesth 2020;125:282–90.
12. Madsen AT, Hojbjerg JA, Sorensen BS, Winther-Larsen A. Day-to-day and within-day biological variation of cell-free DNA. EBioMedicine 2019;49:284–90.
13. Aarsand AK, Roraas T, Fernandez-Calle P, Ricos C, Diaz-Garzon J, Jonker N, et al. The biological variation data critical appraisal checklist: a standard for evaluating studies on biological variation. Clin Chem 2018;64:501–14.
14. Bartlett WA, Braga F, Carobene A, Coskun A, Prusa R, Fernandez-Calle P, et al. A checklist for critical appraisal of studies of biological variation. Clin Chem Lab Med 2015;53:879–85.
15. Braga F, Panteghini M. Generation of data on within-subject biological variation in laboratory medicine: an update. Crit Rev Clin Lab Sci 2016;53:313–25.
16. Hviid CVB, Madsen AT, Winther-Larsen A. Biological variation of serum neurofilament light chain. Clin Chem Lab Med 2021;2360: 569–75.
17. Rissin DM, Kan CW, Campbell TG, Howes SC, Fournier DR, Song L, et al. Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol 2010;28:595–9.
18. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. Crit Rev Clin Lab Sci 1989;27:409–37.
19. Roraas T, Stove B, Petersen PH, Sandberg S. Biological variation: evaluation of methods for constructing confidence intervals for estimates of within-person biological variation for different distributions of the within-person effect. Clin Chim Acta 2017; 468:166–73.
20. Fokkema MR, Herrmann Z, Muskiet FA, Moecks J. Reference change values for brain natriuretic peptides revisited. Clin Chem 2006;52:1602–3.
21. Carobene A, Guerra E, Locatelli M, Ceriotti F, Sandberg S, Fernandez-Calle P, et al. Providing correct estimates of biological variation-not an easy task. The example of S100-beta protein and neuron-specific enolase. Clin Chem 2018;64:1537–9.
22. Hviid CVB, Knudsen CS, Parkner T. Reference interval and preanalytical properties of serum neurofilament light chain in Scandinavian adults. Scand J Clin Lab Invest 2020;80: 291–5.
23. Mattila OS, Ashton NJ, Blennow K, Zetterberg H, Harve-Rytsala H, Pihlasviita S, et al. Ultra-early differential diagnosis of acute cerebral ischemia and hemorrhagic stroke by measuring the prehospital release rate of GFAP. Clin Chem 2021;67:1361–72.
24. Benedict C, Blennow K, Zetterberg H, Cedernaes J. Effects of acute sleep loss on diurnal plasma dynamics of CNS health biomarkers in young men. Neurology 2020;94:e1181–e9.

25. Mestre H, Mori Y, Nedergaard M. The brain's glymphatic system: current controversies. Trends Neurosci 2020;43:458–66.
26. Holth JK, Fritschi SK, Wang C, Pedersen NP, Cirrito JR, Mahan TE, et al. The sleep-wake cycle regulates brain interstitial fluid tau in mice and CSF tau in humans. Science 2019;363:880–4.
27. Shokri-Kojori E, Wang GJ, Wiers CE, Demiral SB, Guo M, Kim SW, et al. Beta-Amyloid accumulation in the human brain after one night of sleep deprivation. Proc Natl Acad Sci U S A 2018;115:4483–8.
28. Huang Y, Potter R, Sigurdson W, Kasten T, Connors R, Morris JC, et al. Beta-amyloid dynamics in human plasma. Arch Neurol 2012; 69:1591–7.
29. Jurga AM, Paleczna M, Kadluczka J, Kuter KZ. Beyond the GFAP-astrocyte protein markers in the brain. Biomolecules 2021; 1411:1361.
30. McKee CA, Lananna BV, Musiek ES. Circadian regulation of astrocyte function: implications for alzheimer's disease. Cell Mol Life Sci 2020;77:1049–58.

Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2022-0480).