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Article

Age-Specific Control and Alzheimer Disease Reference Curves and *z*-Scores for Glial Fibrillary Acidic Protein in Blood

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BACKGROUND: Serum glial fibrillary acidic protein (GFAP) is a biomarker for astrocytic injury and astrogliosis. Concentrations are elevated in numerous neurological disorders, including a pronounced increase in Alzheimer disease (AD). However, GFAP levels in the serum also increase with age. Consequently, the integration of GFAP levels into clinical routine and their interpretation demands age-adjusted reference values.

METHODS: Serum from 1273 subjects (952 noninflammatory and nonneurodegenerative neurological controls and 321 subjects with AD) was analyzed for GFAP using the microfluidic Ella system. Age-dependent serum GFAP reference values were estimated by additive quantile regression analysis and visualized with percentiles and *z*-scores.

RESULTS: AD exhibited elevated serum GFAP levels in comparison to control patients ($P < 0.0001$). This remained the case when the newly generated age-corrected *z*-scores were applied ($P < 0.0001$). In the control cohort, a nonlinear elevation of serum GFAP with increasing age was observed (Spearman correlation coefficient 0.62, 95% CI 0.58–0.66, $P < 0.0001$). In contrast, the AD cohort exhibited a more linear increase (0.16, 95% CI 0.05–0.26, $P = 0.004$). Age-dependent cutoffs for serum GFAP were determined for different AD age groups. The calculated areas under the curve (AUCs; 0.97) demonstrated excellent diagnostic test performance in the early-onset age group. This effect was less marked in the elderly subjects (AUC 0.72).

CONCLUSIONS: Our novel GFAP *z*-scores enable the integration and interpretation of serum GFAP levels in clinical practice, moving from the group to individual level. They support both intra- and interindividual interpretation of single GFAP levels in neurological diseases with astrocytic pathology, including an accurate discrimination of AD.

Introduction

Glial fibrillary acidic protein (GFAP) is a type III intermediate filament almost exclusively expressed in astrocytes in the central nervous system. GFAP is crucial for the mechanical strength of astrocytes and several of their functions, such as the regulation of the blood-brain barrier (1). In the context of astrogliosis, a process, for example, observed following neurodegeneration and neuronal death, there is an increase in GFAP expression. In addition to the normal turnover, following astrocytic injury, GFAP is released into the extracellular space, subsequently reaching the cerebrospinal fluid (CSF) and ultimately the bloodstream (2). In both matrices, CSF and blood, GFAP can be measured using different proteomics approaches, including mass spectrometry, as well as immunoassays such as ELISA, Simoa, and microfluidic assays like Ella (3). Given the expression pattern of GFAP, levels in the CSF are higher than in the blood, which presents a more challenging analytical environment due to strong matrix effects. Nevertheless, numerous studies have consistently demonstrated that blood GFAP exhibits superior discriminatory capabilities between diseases (3–5). It is hypothesized that this may be attributed to a partially direct release of GFAP through the astrocytic endfeet into blood vessels within the central nervous system (6, 7). One neurological condition in which blood GFAP levels are markedly

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elevated is Alzheimer disease (AD) (5, 8, 9). Moreover, in genetic AD patients' blood, GFAP levels appear to increase more than 10 years before the clinical onset of symptoms, suggesting that they may also have prognostic value (10, 11). Additionally, studies have demonstrated its utility as a progression marker from AD with mild cognitive impairment to AD dementia (12, 13). In therapeutic studies targeting Aβ, GFAP blood levels have been observed to decrease after several months of treatment, potentially reflecting a reduction in astrocytic damage or astrogliosis (14, 15). Consequently, GFAP may also prove to be a highly valuable treatment monitoring marker in a clinical setting on an individual level. However, studies have consistently demonstrated that age is correlated with GFAP levels in both CSF and blood (13, 16–18). This renders the interpretation of GFAP levels in clinical routine more challenging in the absence of age-dependent reference values. Ways of describing those reference values can be as traditional percentiles or *z*-scores. A *z*-score quantifies how far a given value lies from the average of its dataset in terms of SDs. Converting the absolute values into age-corrected *z*-scores enables direct comparisons, making it simple to spot unusually high or low observations without the need for age-specific curves.

In this study, nearly 1000 serum samples of control patients without neuroinflammatory and neurodegenerative diseases from a broad age range were used to establish age-dependent reference curves, absolute values, and *z*-scores for serum GFAP. Additionally, the same methodology was applied to a cohort of over 300 AD samples, enabling the estimation of age-dependent cutoffs for the diagnosis of AD.

Material and Methods

PATIENTS

For this study, there were 2 sources for control patients: (*a*) the population-based ALS Swabian registry, which also includes controls, and (*b*) patients seen at the Department of Neurology at the University Hospital Ulm, which were classified as controls (see later discussion).

From the population-based ALS Swabian register, we analyzed 577 participants enrolled as controls, which were sampled randomly from the general population (ethics votes No. 11/10, No. B-F-2010-062, and No. 7/11 300). The study design and recruitment procedures of the ALS Swabian register have been described previously (19–22).

To make the additive quantile regression analysis for the generation of age-specific GFAP percentiles and *z*-scores more accurate, we additionally measured samples from 424 patients seen at Ulm University Hospital between 2014 and 2023 (for selection and inclusion/exclusion criteria, see the flow chart in Supplemental Fig. 1). The patients were selected through convenience sampling. For the 424 patients seen at Ulm University Hospital, acute neuroinflammation of the central nervous system was ruled out by CSF analysis (normal cell count; no evidence of intrathecal immunoglobulin synthesis). In addition, the patients did not show clinical or radiological signs of chronic neuroinflammation and neurodegeneration. For more details on the diagnoses, see Supplemental Table 1. Due to GFAP levels below the lower limit of detection, 49 control subjects were excluded from further analysis.

The 324 AD patients were clinically diagnosed at Ulm University Hospital according to the International Working Group-2 criteria (23) and sampled between 2009 and 2023 (see flow chart in Supplemental Fig. 1). Additionally, all CSF AD samples were retrospectively examined for the amyloid-beta/tau/neurodegeneration (ATN) core markers [A: CSF Aβ1–42 to Aβ1–40 ratio, T: CSF phospho-tau (p-tau) 181, and N: CSF total-tau (t-tau)], to be able to classify them according to the ATN system (24). All ADs were A + with 270 patients A + T + (270 A + T + N + and 0 A + T + N−), and 51 A + T− (21 A + T-N + and 30 A + T-N−) (3 patients were excluded due to GFAP levels below the lower limit of detection). Control and AD patients with an acute or chronic renal insufficiency were excluded from the study.

The examination was approved by the local ethics committee (approval number Ulm 20/10) and conducted following the Declaration of Helsinki. All participants gave their written informed consent to participate in the study.

SAMPLING AND BIOMARKER MEASUREMENTS

Blood samples were collected by venous sampling and centrifuged at 2000*g* for 10 min, and the extracted serum was aliquoted and frozen on the same day at −80°C. All serum samples were stored in polypropylene tubes.

For serum GFAP quantification, we applied the microfluidic Ella platform (BioTechne) using the second-generation GFAP cartridges, which were recently technically and clinically validated (25). The analyses were performed according to the manufacturer's instructions. Intra- and interassay variations for 2 serum QCs measured in duplicates on each cartridge were below 20%.

ATN markers were analyzed using the Lumipulse G 600II platform (Fujirebio).

STATISTICAL ANALYSIS

The distribution of data was assessed visually with density plots and statistically using the Shapiro–Wilk-test in

Table 1. Study cohort.[a]

N	Control group n = 952	AD patients n = 321
Age (years), median [Q1, Q3]	61 (44–71)	73 (67–78)
Female (%)	477 (50.1)[b]	188 (58.5)[c]
Serum GFAP (pg/mL), median [Q1, Q3]	4.3 (2.6–6.8)	15.1 (10.8–20.4)
MMSE median [Q1, Q3]		23 (19–26)
CDR-SOB median [Q1, Q3]		3.5 (2–5)

Abbreviations: CDR-SOB, Clinical Dementia Rating Sum of Boxes; MMSE, Mini Mental State Examination.
[a]Data are shown as median and interquartile range or N and percentage.
[b]Female control patients were younger than males ($P = 0.004$).
[c]No age difference was found between female and male ADs ($P = 0.97$).

the whole group, for female and male as well as in the different age groups. Because data were non-gaussian, nonparametric tests were used.

Due to a nonlinear relation between age and serum GFAP levels, additive quantile regression analysis was performed based on the control population to assess the effect of age on GFAP concentrations. In the quantile regression model, the age-specific percentiles were thereby determined by using age as the predictor (26). According to this analysis, we estimated the *z*-scores for the control group. We also applied this model to estimate the age-specific percentiles for the AD group. For the analysis, the "quantreg" and "qgam" packages in R were applied (for more details on the model, see the Supplemental Material).

For a 2-group comparison, the Mann–Whitney *U*-test (2-tailed) was applied, and for more groups, the Kruskal–Wallis test followed by the Dunn multiple comparisons test was applied, with a $P < 0.05$ indicative of statistically significant results. For the discrimination between controls and AD patients, the estimation of cut-offs using receiver operating characteristic (ROC) analysis was applied. The maximization of the Youden index was used for optimization of the cut-off levels. For association testing between serum GFAP and other parameters, Spearman rank correlations (ρ) were applied. All analyses were performed applying 2-sided tests. The visualization and analysis were performed with RStudio V. 4.3.1 and GraphPad Prism V.10.3.1 (GraphPad).

Results

The demographic as well as GFAP serum values of the control and AD cohort are shown in Table 1. For the establishment of age-dependent control and AD GFAP reference curves and values, 952 controls and 321 AD patient samples were used.

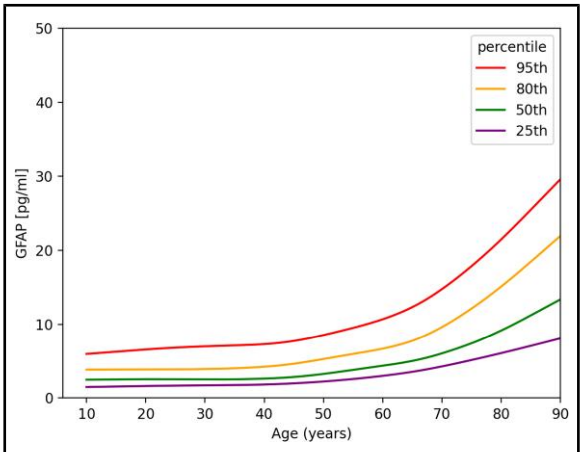

Fig. 1. Serum GFAP age-dependent control reference curves. The serum GFAP percentiles dependent on age. For the modeling, additive quantile regression analysis of 951 control patients was applied. Color figure available at clinchem.org.

SERUM GFAP LEVELS IN THE CONTROL COHORT

Figure 1A depicts the GFAP serum reference curves from the 25th to the 95th percentile in the control cohort, stratified by age. The serum GFAP values demonstrate an increase with age, commencing at approximately 50 years. The median (interquartile range) for the group of patients below 50 years was 2.6 pg/mL (1.8–3.6 pg/mL), while the patients above 80 years of age exhibited a significantly higher median of 11.7 pg/mL (7.1–17.0 pg/mL). Using the 50th percentile, we determined the average GFAP elevation per year. We estimated an increase of 0.63% below 50 years and 3.58% per year above 50 years of age. The correlation between age and serum GFAP levels was found to be moderate to strong, with a Spearman ρ of 0.62

Table 2. Age-specific 50% and 95% GFAP percentiles in serum of control and AD patients.

Age (years)	Control 50th percentile [pg/mL]	Control 95th percentile [pg/mL]	AD 50th percentile [pg/mL]	AD 95th percentile [pg/mL]
20	2.48	6.07	NA	NA
25	2.49	6.37	NA	NA
30	2.51	6.67	NA	NA
35	2.55	6.97	NA	NA
40	2.66	7.32	10.8	27.6
45	2.87	7.75	11.4	28.3
50	3.24	8.38	12.1	29.0
55	3.77	9.27	12.8	29.6
60	4.35	10.4	13.5	30.3
65	5.00	12.0	14.1	30.9
70	6.03	14.4	14.8	31.6
75	7.32	17.6	15.5	32.2
80	8.95	21.4	16.1	32.9
85	11.4	26.5	16.8	33.5

Abbreviation: NA, not applicable.

(95% CI 0.58–0.66), $P < 0.0001$. Table 2 presents the serum GFAP concentrations corresponding to the 50th and 95th percentiles for various age groups. *z*-score values can be found in Supplemental Table 2. Including patients below the lower limit of detection in the model led to little difference in the outcome (see Supplemental Materials).

We did not find an association between sample freezer time and serum GFAP values ($\rho = 0.04$ (95% CI −0.03–0.10), $P = 0.31$) in the control group. Furthermore, we found no significant difference between serum GFAP levels in female and male control patients when looking at the whole control group ($P = 0.07$). Results for GFAP levels stratified by age and sex as well as the n for the different age groups can be found in the Supplemental Materials.

SERUM GFAP LEVELS IN THE AD COHORT

In the AD cohort, GFAP values were significantly increased compared to controls in all AD, ATN + ADs, and ADs with a A + T− (N+/−) CSF biomarker profile (Fig. 2A and B). We detected no difference between the AD groups. Figure 2C illustrates that GFAP values were also elevated in the different AD groups when age-corrected *z*-scores, based on the regression analysis of the control cohort, were applied. For the discrimination between control and AD patients, ROC analysis depicted an area under the curve (AUC) of 0.93 (95% CI 0.91–0.94) for all ADs, 0.93 (95% CI 0.92–0.95) for the AD A + T + N + , and 0.92 (95% CI 0.89–0.95) for the AD A + T−(N+/−) group (Fig. 2D). The optimal cut-off for the whole AD group was determined to be at 8.1 pg/mL with a diagnostic sensitivity of 92% (95% CI 88%–94%) and specificity of 84% (95% CI 80%–85%). However, as a high diagnostic specificity is highly desirable in routine analysis, we also determined the cut-off for a diagnostic specificity of 95%, which was found to be 14.0 pg/mL with a diagnostic sensitivity of 55% (95% CI 50%–61%). Using *z*-scores instead of absolute values for the ROC analysis of the whole AD group, the AUC was 0.87 (95% CI 0.85–0.89). The Youden index maximum revealed an optimal *z*-score cut-off of 0.59 with a diagnostic sensitivity of 85% (95% CI 80%–88%) and specificity of 75% (95% CI 71%–77%). The cut-off for a diagnostic specificity of 95% was at a *z*-score cut-off of 1.5 (sensitivity 50%; 95% CI 45%–56%) (see the Supplemental Material for further details).

Moreover, the availability of control samples across a wide age range enabled a comparison of serum GFAP concentrations across different age groups (Fig. 3A). All AD patient groups between the ages of 50 and 90 exhibited significantly elevated levels in comparison to the corresponding age control group. The results of the ROC analysis indicated that the youngest patient group with ADs and controls between the ages of 51 and 60 exhibited the highest AUC. Subsequently, the AUC values decreased with increasing age of the stratified age groups (Fig. 3B). The optimal and cut-off values for a diagnostic specificity of 95% for each age group, along

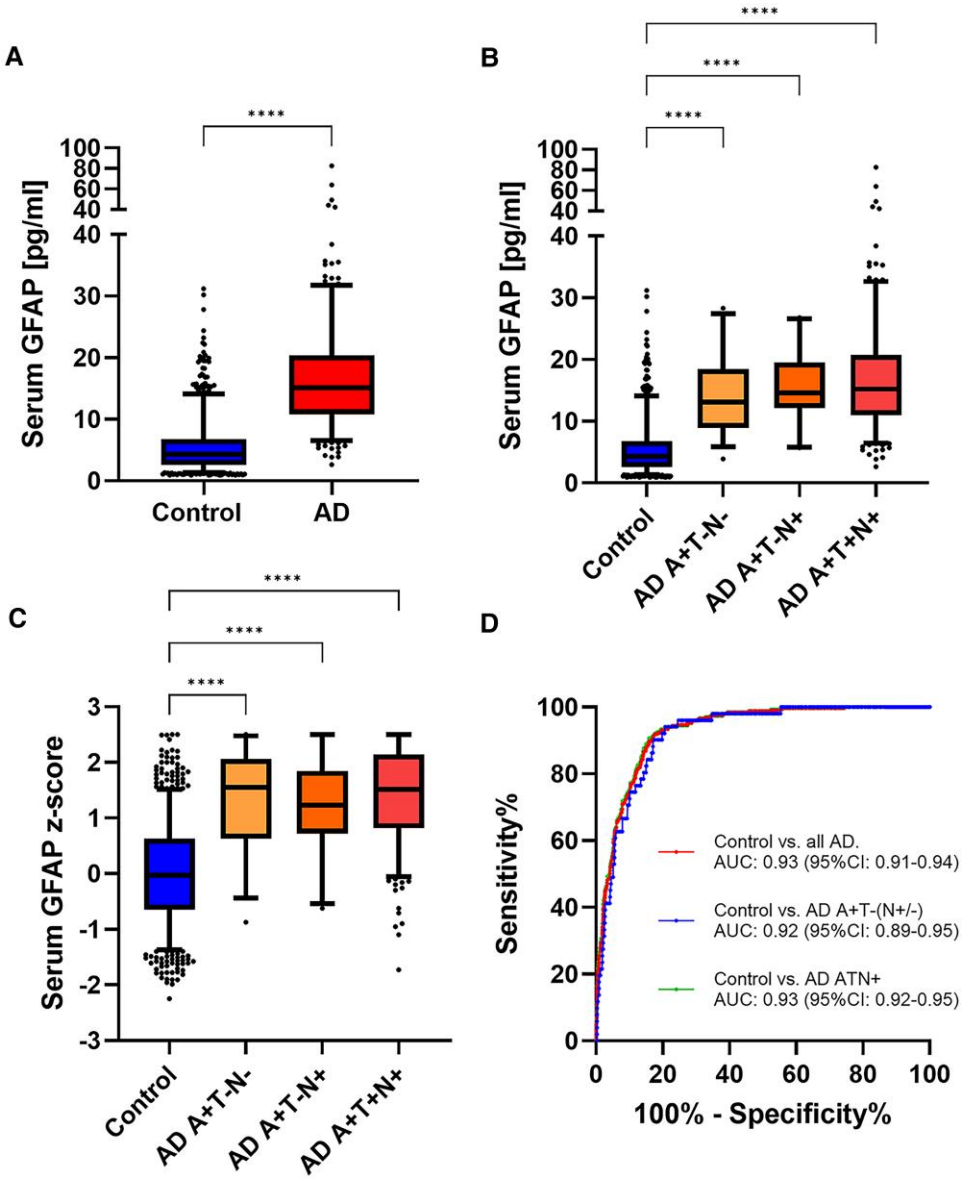

Fig. 2. Serum GFAP analysis in the AD cohort. (A), The serum GFAP comparison between control and all AD patients with significantly higher levels in the AD group; (B), The comparisons with the AD group stratified according to CSF ATN markers. All groups demonstrated markedly increased serum GFAP concentrations compared to controls. The same is true when age-corrected *z*-scores (C) are applied. (D), The discriminating potential of serum GFAP for controls vs all ADs, AD A + T +, and A + T– is illustrated. The ROC analysis yielded nearly the same high AUCs for all comparisons. Color figure available at clinchem.org.

with the corresponding sensitivity and specificity values, can be found in Supplemental Table 6. Additionally, Supplemental Tables 7 to 9 show the results for the same analysis using *z*-scores.

Subsequently, we conducted a more detailed examination of how age affects GFAP levels within the AD cohort. Figure 4A illustrates the linear elevation of GFAP concentrations with increasing age, as demonstrated by quantile regression analysis. Table 2 presents the serum GFAP concentrations for the 50th and 95th percentiles for different age groups in the AD cohort. The correlation coefficient ρ for GFAP and age in the

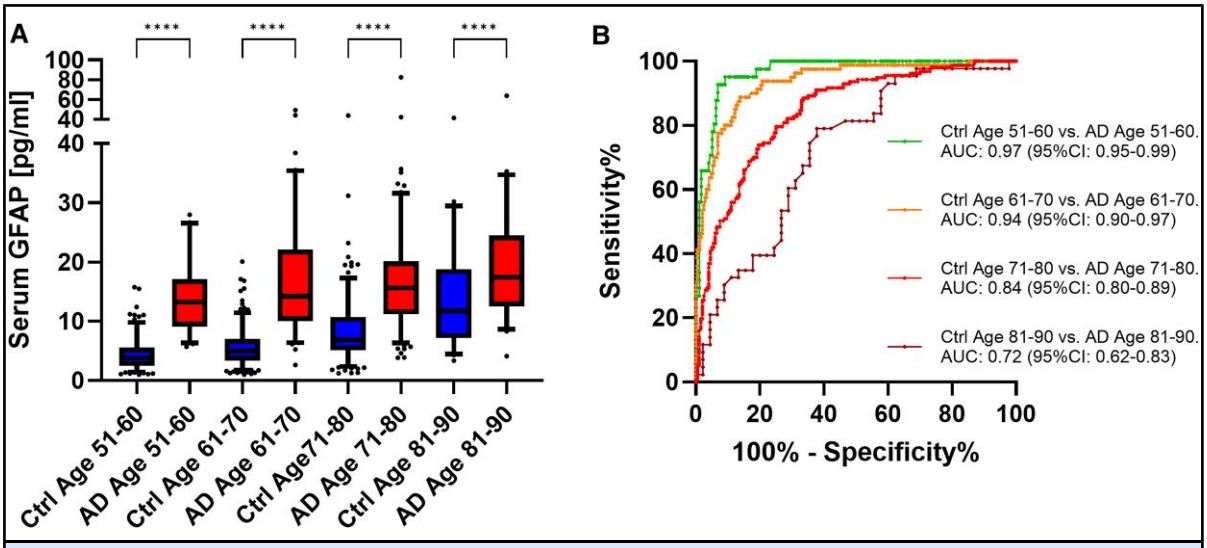

Fig. 3. Comparison of serum GFAP stratified by age groups of 10 years. (A), Serum GFAP levels stratified according to age decade compared between control and AD patients. All age groups show significantly increased levels in AD; (B), The serum GFAP ROC analysis between the control and corresponding AD age groups. The AUCs are highest in the younger age groups and decline with older age (51–60: Ctrl n = 175, AD n = 41; 61–70: Ctrl n = 246, AD n = 80; 71–80: Ctrl n= 200, AD n = 157; 81–90: Ctrl n = 45, AD n = 43). Abbreviation: Ctrl, control. Color figure available at clinchem.org.

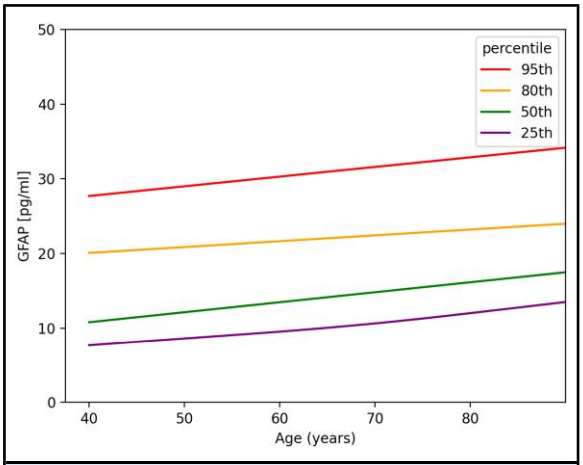

Fig. 4. Serum GFAP age-dependent AD reference curves. Serum GFAP percentiles are dependent on age of the AD patient cohort. For the modeling, additive quantile regression analysis was applied. Color figure available at clinchem.org.

AD cohort was determined to be 0.16 (95% CI 0.05–0.26, $P = 0.004$). In the whole AD group, there was a trend to higher levels in female compared to male patients, which was, however, not statistically significant ($P = 0.07$). The same was true when the AD group was stratified according to age (see Supplemental Materials).

CORRELATIONS WITH ATN MARKERS

The correlation between serum GFAP levels and CSF t-tau ($\rho = 0.14$, 95% CI 0.03–0.25, $P = 0.011$) and p-tau 181 ($\rho = 0.14$, 95% CI 0.03–0.24, $P = 0.012$) in the AD group was found to be weak. No significant differences were observed in CSF t-tau and p-tau levels across the various age groups (Supplemental Fig. 5A and B).

When the AD cohort was divided into patients with mild cognitive impairment and dementia, serum GFAP levels were already elevated in the mild cognitive impairment group compared to controls ($P < 0.0001$) and remained elevated in the AD with dementia group ($P < 0.0001$) (see Supplemental Fig. 7).

Discussion

Serum GFAP is an important fluid astrocytic biomarker that is increasingly being recognized as a valuable tool for routine applications in clinical settings. Nevertheless, in order to interpret serum GFAP levels in routine analysis, it is of utmost importance to account for the observed increase in levels with advancing age, a

phenomenon that has been consistently demonstrated in numerous publications. This study addresses this issue by estimating and graphically displaying age-corrected reference values using absolute values and *z*-scores. Given the elevated serum GFAP levels observed in AD patients in the literature, we also determined age-reference values for AD and calculated age-dependent cut-off levels.

The results demonstrate a clear increase in serum GFAP levels with age, which is nevertheless less pronounced than that observed for the neurofilament light chain protein, for which several age-reference studies have been conducted (26–28). The available data on serum GFAP reference values, however, is limited. Studies with a smaller number of adults conducted by Danish and Canadian colleagues examined GFAP reference values using the Simoa technology (29, 30). They also demonstrate an increase with age starting around 50 years of age, which is less pronounced than for neurofilament light chain protein. It should be noted, however, that the absolute values of these studies and our data are not directly comparable, and no *z*-scores were reported. The application of *z*-scores, determined in our study, facilitates the interpretation of the data and renders it more independent of the platform utilized to measure serum GFAP levels. Additionally, the use of age-corrected *z*-scores for GFAP, defined as the number of SDs a single GFAP value is above or below the mean GFAP level for a given age, offers further advantages, including a normal distribution and the potential for negative values.

The significance of age-reference values for the interpretation of GFAP levels can be illustrated by a straightforward example. According to our data, a serum GFAP measurement of 6 pg/mL is considered normal for patients at age 70 years and elevated for patients at age 20 years. The use of *z*-scores allows for the direct observation of this distinction without the need for a table or graphic. For instance, a serum GFAP value of 6 pg/mL corresponds to a *z*-score of −0.02 for an age of 70, indicating that the serum GFAP value of 6 pg/mL is nearly the median of this age stage. However, a serum GFAP concentration of 6 pg/mL corresponds to a *z*-score of 1.63 at the age of 20, indicating a level that is more than 1.5 times the SD above the serum GFAP median level at this age, rendering it clearly elevated. (For a possible interpretation of *z*-score GFAP values, see the Supplemental Material.) For the application of our reference curves and *z*-scores, it is of utmost importance to control for batch effects that could lead to variances between GFAP cartridges. How we controlled for differences between lots can be found in the Supplemental Material.

The GFAP analysis in the AD cohort corroborates the findings of previous studies that have demonstrated that serum GFAP levels are significantly elevated in AD patients compared to controls (5, 8, 9). In addition to elevated levels in ATN-positive AD patients, we also found a significant increase in AD patients only positive for the CSF beta amyloid 42 to 40 ratio. This finding is consistent with the results of previous studies that also identified elevated serum GFAP levels in A + T– patients (4, 16). Furthermore, we demonstrate that the elevation in serum GFAP observed in AD patients is confirmed when age-corrected *z*-scores are applied. Notably, the AUC derived from age-corrected *z*-scores was, at 0.87, still high but lower than that obtained using absolute values. This discrepancy likely reflects the influence of age as a confounding variable. In particular, the elevated AUC observed with absolute values may be partially attributable to the older age distribution within the AD group. By incorporating age normalization, the *z*-score approach mitigates this bias. The observed AUCs between 0.72 and 0.97, depending on the age group, also confirm the literature, which reports AUCs between 0.79 and 0.93 (8, 13, 31). The lower AUCs in the older age groups result from higher blood GFAP levels in the control patients in these age groups. This elevation is probably mostly due to an aging effect driven by increased astrogliosis and compromised integrity of the blood-brain barrier (8). However, as it has been shown that GFAP levels could already increase in presymptomatic disease stages (11), it cannot be ruled out that a few of the elderly patients are in a clinically and radiologically so far not detectable disease stage. Nonetheless, the analysis of a large number of control patients across a wide age range enabled the establishment of age-specific cut-offs for AD. They can assist in differentiating between elevated serum GFAP levels resulting from disease-specific astrocytic injury or astrocytosis in AD and those caused by normal aging effects in the elderly population.

Our findings for the age-reference curves for the AD cohort indicated a more linear increase compared to the control curves. In the literature, there are no serum GFAP reference curves available for AD to compare with. However, neurofilament light chain protein was also examined in AD, and a similar pattern of a more gradual linear increase was observed (26). In a disease cohort such as AD, it is necessary to determine whether the elevation of serum GFAP with increasing age is due to the effects of aging or to a more severe disease pathology in older age groups, which may result in increased GFAP levels in the blood. To address this question, we analyzed the correlation between serum GFAP and CSF markers, particularly total and p-tau, which are known to be associated with atrophy and disease intensity (32–34). The weak correlation between serum GFAP and CSF t-tau and p-tau is in accordance with the findings of other studies, which indicate that serum GFAP is not a marker of tau pathology (35). In any case, the CSF t-tau and

p-tau 181 levels are not different between younger and older AD patients, indicating a degenerative process of comparable severity in the different age stages. The observed increase in blood GFAP in older AD patients might therefore also be an effect of aging as discussed earlier.

The principal strength of our study is the analysis of nearly 1000 control subjects for the establishment of age-dependent reference values and *z*-scores. Furthermore, the generation of *z*-scores facilitates straightforward interpretation of the results and renders the interpretation independent of the analytical platform. Additionally, the ATN-characterized AD group permitted the generation of age-specific cut-offs, which could prove invaluable in clinical routine analysis. A potential limitation of this study is the inapplicability of the results for patients with renal dysfunction and the relatively small subgroups of AD patients included. Future studies could aim to recruit a larger number of AD patients to improve the accuracy of very high or low *z*-scores.

In conclusion, our study offers age-dependent reference curves, values, and *z*-scores for serum GFAP, which could greatly aid in clinical practice by supporting the interpretation of individual GFAP levels and facilitating the integration of GFAP analysis into clinical reports. The reference values are applicable to any clinical scenario exploring active astrocytic changes in neurological diseases. Additionally, we provide age-specific serum GFAP cut-offs tailored for ATN-categorized AD patients.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Author Declaration: A version of this paper was previously posted as a preprint on medRxiv as https://doi.org/10.1101/2024.12.04.24318285.

Nonstandard Abbreviations: GFAP, glial fibrillary acidic protein; CSF, cerebrospinal fluid; AD, Alzheimer disease; ATN, amyloid-beta/tau/neurodegeneration; p-tau, phospho-tau; t-tau, total-tau; ROC, receiver operating characteristics; AUC, area under the curve.

Author Contributions: *The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.*

Steffen Halbgebauer (Conceptualization-Equal, Methodology-Equal, Project administration-Equal, Resources-Equal, Supervision-Equal, Validation-Equal, Writing—original draft-Lead, Writing—review & editing-Equal), Badrieh Fazeli (Methodology-Equal, Writing—review & editing-Equal), Veronika Klose (Data curation-Equal, Software-Equal, Writing—review & editing-Equal), Gabriele Nagel (Resources-Equal, Writing—review & editing-Equal), Angela Rosenbohm (Resources-Equal, Writing—review & editing-Equal), Dietrich Rothenbacher (Resources-Equal, Writing—review & editing-Equal), Franziska Bachhuber (Methodology-Equal, Writing—review & editing-Equal), Sarah Jesse (Resources-Equal, Writing—review & editing-Equal), Markus Otto (Resources-Equal, Writing—review & editing-Equal), G. Bernhard Landwehrmeyer (Resources-Equal, Writing—review & editing-Equal), Ahmed Abdelhak (Resources-Equal, Writing—review & editing-Equal), Axel Petzold (Resources-Equal, Writing—review & editing-Equal), Albert C. Ludolph (Resources-Equal, Writing—review & editing-Equal), and Hayrettin Tumani (Conceptualization-Equal, Funding acquisition-Lead, Project administration-Equal, Resources-Lead, Supervision-Lead, Writing—review & editing-Equal)

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