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Glial Fibrillary Acidic Protein Serum Levels Distinguish between Intracerebral Hemorrhage and Cerebral Ischemia in the Early Phase of Stroke

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BACKGROUND: Recent studies have suggested that glial fibrillary acidic protein (GFAP) serum concentrations distinguish between intracerebral hemorrhage (ICH) and ischemic stroke (IS) shortly after symptom onset. In this prospective multicenter trial we validated GFAP in an independent patient cohort and assessed the quantitative relationship between GFAP release, bleeding size, and localization.

METHODS: We included patients with a persistent neurological deficit (NIH Stroke Scale ≥4) suggestive of stroke within 6 h of symptom onset. Blood samples were drawn at hospital admission. GFAP serum concentrations were measured using an electrochemiluminometric immunoassay. Primary endpoint was the final diagnosis established at hospital discharge (ICH, IS, or stroke mimic).

RESULTS: 202 patients were included (45 with ICH, 146 with IS, 11 stroke mimics). GFAP concentrations were significantly higher in ICH than in IS patients [median (interquartile range) 0.16 μg/L (0.04–3.27) vs 0.01 μg/L (0.01–0.01), $P < 0.001$]. A GFAP cutoff of 0.03 μg/L provided a sensitivity of 77.8% and a specificity of 94.2% in distinguishing ICH from IS and stroke mimics [ROC analysis area under the curve 0.872 (95% CI, 0.802–0.942), $P < 0.001$]. GFAP serum concentrations were positively correlated with ICH volume. Lobar ICH volumes were larger and thus associated with higher GFAP concentrations as compared to deep ICH.

CONCLUSIONS: Serum GFAP was confirmed to be a biomarker indicating ICH in patients presenting with acute stroke symptoms. Very small ICH may be missed owing to less tissue destruction.

Worldwide, stroke is the third leading cause of death and the leading cause of long-term disability *(1)*. Apart from the functional and emotional burden for the affected patient, the disease has significant economic impact *(1)*. Hence, increasing the efficacy of stroke treatment with the goal of improving long-term outcomes and reducing healthcare costs remains a challenge of utmost importance. This holds particularly true in the industrialized nations where stroke incidence is expected to increase with an anticipated growth of average life expectancy.

Regardless of stroke subtype, treatment of the affected patient should be initiated as early as possible *(2, 3)*. Metaanalyses of randomized thrombolysis studies as well as endovascular therapy studies have shown that a shorter time interval between symptom onset and treatment translates into more favorable outcomes *(3, 4)*. In intracerebral hemorrhage (ICH),[10] early blood pressure control is recommended immediately after symptom onset to prevent recurrent or ongoing bleeding *(2)*.

Hitherto, brain imaging has been the diagnostic standard to differentiate between ICH and ischemic stroke (IS) *(5)*. Using brain imaging requires the patient to be taken to a computed tomography (CT)-equipped hospital, and using a CT-equipped hospital in turn increases the time to treatment *(6)*. Thus, a biomarker test rapidly distinguishing between patients with IS and ICH

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[10] Nonstandard abbreviations: ICH, intracerebral hemorrhage; IGNITE, Initiative of German Neurointensive Trial Engagement; IS, ischemic stroke; CT, computed tomography; GFAP, glial fibrillary acidic protein; BE FAST I, Biomarker for Rapid Diagnosis of Hemispheric Stroke I; BE FAST II, Biomarker for Rapid Diagnosis of Hemispheric Stroke II; NIHSS, NIH Stroke Scale; VKA, vitamin K antagonists; LoB, limit of blank; LoD, limit of detection; TPA, tripropylamine; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

could be valuable, and ideally will steer development of precise, accurate point-of-care assays in future *(7)*.

One currently investigated biomarker target in this context is the serum concentration of glial fibrillary acidic protein (GFAP), an astroglial intermediate filament protein. In ICH, GFAP is released rapidly from brain cells into the blood stream secondary to the immediate glial destruction induced by the expanding hematoma *(8)*. In contrast, in IS, GFAP release does not occur before 6–12 h after vessel occlusion, as necrosis and cellular disintegration are typically not present in the first hours of IS *(5, 9)*. Following smaller proof of concept trials *(9)*, the first prospective multicenter study [Biomarker for Rapid Diagnosis of Hemispheric Stroke I (BE FAST I)] was published in 2012 *(10)*. GFAP was found to correctly identify approximately 80% of patients with ICH, whereas IS patients had GFAP values below the cutoff level in about 97% of cases. The present study was designed to validate these findings in an independent cohort of stroke patients with slightly modified inclusion criteria. Furthermore, it was hypothesized that potential associations between bleed size, localization, and GFAP serum concentrations may explain the lower sensitivity compared to the high specificity in detecting ICH. The current study was designed to investigate these associations in a secondary analysis.

METHODS

The Biomarker for Rapid Diagnosis of Hemispheric Stroke II (BE FAST II) study was designed following the guidelines of the Standards for Reporting of Diagnostic Accuracy initiative *(11)*. The study protocol was approved by the institutional review board of the Goethe-University Hospital/Frankfurt am Main and of the participating hospitals (see below). Written informed consent was obtained from all patients/legal representatives before ultimate inclusion in the study. However, as per study protocol, before obtaining informed consent, an aliquot of the routine blood sample drawn upon hospital admission was taken for study purposes.

STUDY DESIGN

BE FAST II is a multicenter study including 8 secondary and tertiary stroke centers in Germany (Department of Neurology, Goethe-University, Frankfurt am Main; Charité Center for Stroke Research Berlin, Berlin; Department of Neurology, Hochtaunus-Kliniken, Bad Homburg; Department of Neurology, Horst Schmidt Klinikum, Wiesbaden; Department of Neurology, Universitätsklinikum Münster; Department of Neurology, Universitätsklinikum Carl Gustav Carus, Dresden; Department of Neurology, Klinikum Herford, Herford; Department of Neurology, Universitätsklinikum Halle/Saale).

Between April 2012 and September 2013, we screened all patients who were admitted with clinical symptoms suggestive of acute stroke. The 3 inclusion criteria were (*a*) age $\geq$18 years, (*b*) time from symptom onset to hospital admission $<$6 h, and (*c*) NIH Stroke Scale (NIHSS) score at hospital admission $\geq$4 points. The determination of symptom onset time was based on the information provided by patient, next-of-kin, or other witnesses ("when was the patient last known to be well?"). The 3 exclusion criteria were (*a*) stroke (including IS and ICH) or transient ischemic attack in the past 3 months, (*b*) traumatic brain injury in the past 3 months (including head concussion at symptom onset of the actual stroke) *(12)*, and (*c*) brain tumor at any time in the past medical history (recent data suggested that GFAP serum concentrations are markedly increased in patients with malignant glioma) *(13)*. The following clinical baseline variables were recorded: age, sex, admission NIHSS, presence of a hemiparesis at hospital admission (i.e., unilateral paresis of arms and leg), presence of clinical signs indicating hemispheric involvement (aphasia, neglect, homonymous hemianopia, forced gaze deviation, impairment of consciousness), history of arterial hypertension, diabetes or hyperlipidemia (defined according to current guidelines) *(14–16)*, current and prior treatment with vitamin K antagonists (VKA) or antiplatelet treatment, and time interval between symptom onset and hospital admission. The primary endpoint of the study was the final diagnosis at hospital discharge, categorized as IS (including transient ischemic attack), ICH, or stroke mimic. Stroke mimics were defined as conditions clinically imitating the symptoms of acute stroke (such as epileptic seizures with hemiparesis in the postictal period, otogenic vertigo, decreased level of consciousness or transient focal neurological deficits secondary to infection, uremia, hypoglycemia, or dehydration). The diagnosis was established according to the *International Classification of Diseases*, 10th revision, on the basis of all available clinical data, brain imaging, laboratory results, and other examinations.

ICH VOLUME QUANTIFICATION

According to study protocol at least 1 brain scan, irrespective of modality (MRI, CT), was required within 24 h of hospital admission. Location of ICH was determined on the first available brain imaging and classified into "lobar" or "deep." Intraventricular hemorrhage expansion, if present, was also documented. ICH volumes were quantified by means of the $(a \times b \times c)/2$ method *(17)*. In patients with IS, infarct sizes were not quantified.

BLOOD SAMPLING

An aliquot (approximately 1 mL) was collected from the routine blood sample obtained upon hospital admission

Table 1. Baseline characteristics of the study population.

	IS	ICH	Stroke mimic	All[a]
n (%)	146 (72.3)	45 (22.3)	11 (5.4)	202 (100.0)
Mean age, years (SD)	74.5 (11.9)	68.4 (15.8)	73.5 (10.0)	73.1 (13.0)
Male, n (%)	73 (50.0)	20 (44.4)	6 (54.5)	99 (49.0)
Median NIHSS, (IQR)	8 (5–16)	12 (7–18)	10 (6–12)	9 (6–16)
Hemiparesis, n (%)	123 (84.2)	36 (80.0)	6 (54.5)	165 (81.7)
Cortical signs, n (%)	98 (67.6)	28 (62.2)	5 (45.5)	131 (65.3)
Hypertension, n (%)	122 (84.1)	34 (75.6)	9 (81.8)	165 (82.1)
Diabetes, n (%)	34 (23.6)	9 (20.0)	4 (36.4)	47 (23.5)
Hypercholesterolemia, n (%)	52 (36.1)	6 (13.6)	4 (36.4)	62 (31.2)
VKA treatment, n (%)	14 (9.7)	8 (17.8)	3 (30.0)	25 (12.6)
Antiplatelet treatment, n (%)	70 (48.6)	11 (24.4)	6 (54.5)	87 (43.5)
Mean time from symptom onset to hospital admission, h:min (SD)	01:54 (01:16)	01:55 (01:32)	01:22 (00:50)	01:53 (01:18)

[a] Because of a few missing values, sums do not always equal 100%.

in a blood tube containing coagulation activating agent (S-Monovette, 4.7 mL, Sarstedt). Blood tubes were routinely centrifuged at 1500–2000 g for 10 min. The supernatant was stored in Eppendorf tubes at −80 °C. As a precautionary measure samples were shipped on dry ice although it has been shown that GFAP is stable for several days at 4 °C. Up to 4 freezing–thawing cycles do not influence GFAP concentrations *(18)*.

GFAP MEASUREMENTS

Serum samples were analyzed at Roche Diagnostics, Penzberg, Germany. An electrochemiluminometric immunoassay was used for the in vitro quantitative determination of GFAP in human serum (Elecsys® GFAP prototype test measured on a cobas® e411 platform). This assay has previously been used in explorative GFAP studies in stroke (including BE FAST I) and other neurological diseases *(10, 13, 19, 20)*. It has been modified for the use in this trial by the selection of monoclonal antibodies with higher affinity to further improve sensitivity for detecting GFAP. Standardization of the GFAP assay remained unchanged (including buffer composition) and was based on the former version. The measuring range of the modified test was 0.02–100 μg/L, repeated measurement precision (5 samples and 3 controls, 21-fold each, 1 calibrated run) was 0.8–6.2 CV%, and between day precision (5 samples and 3 controls, 6 aliquots each, 10 independently calibrated runs, maximum 2 runs per day) was 1.8–5.9% CV. Limit of blank (LoB) was 0.02 μg/L, and limit of detection (LoD) was 0.03 μg/L. Healthy individuals (n = 115 serum samples) revealed GFAP values below 0.03 μg/L in 95% of cases using the modified GFAP assay. The impact of hemolysis on the modified Elecsys GFAP assay was tested and no interference could be observed up to at least 6 g/L of hemoglobin. All scientists involved in the measurements were fully blinded to the clinical data.

The GFAP quantification procedure has been described in detail previously *(10, 13)*. In brief, biotin- and ruthenium-labeled monoclonal GFAP antibodies were combined with 50 μL of serum sample and incubated for 9 min. Streptavidin-coated magnetic microparticles were added, and the mixture was incubated for another 9 min. The reaction mixture was pipetted into the measuring cell where the beads were magnetically captured on an electrode surface. In a washing step, unbound microparticles were removed from the measuring cell. In the last step, voltage was applied to the electrode in the presence of a tripropylamine (TPA)-containing buffer which resulted in an electrochemiluminescent signal that was recorded by a photomultiplier.

STATISTICAL ANALYSIS

Statistical analyses were performed using IBM® SPSS® Statistics, Version 22 (Statistical Package for the Social Sciences). Because GFAP serum concentrations were not normally distributed between individuals, statistical comparisons were made using the nonparametric Mann–Whitney *U* test. Correlation analyses were performed by means of the nonparametric Spearman rank test. The optimal serum GFAP cutoff concentration to distinguish ICH from IS and stroke mimic was calculated using ROC-curve analysis. Sensitivity and specificity measures as well as the positive predictive value (PPV) and the negative predictive value (NPV) were derived from cross-tabulations. Multivariate regression analysis was used to

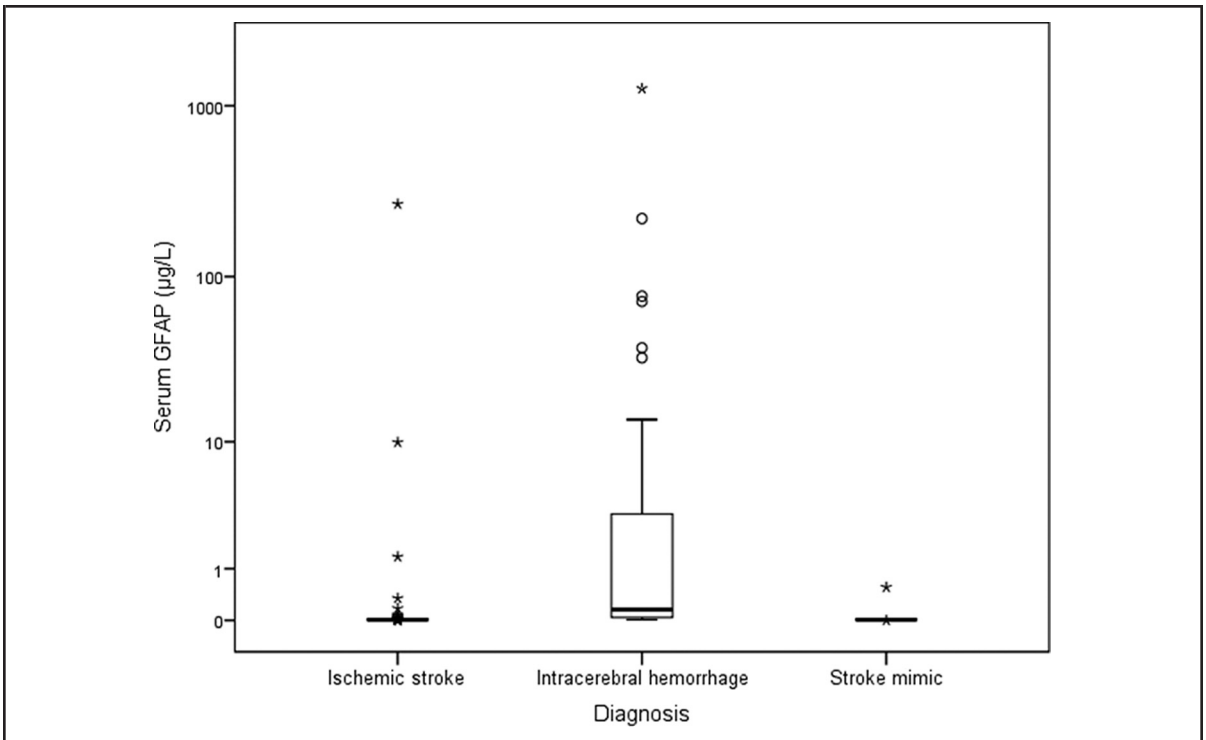

Fig. 1. Box and whisker plots visualizing the distribution of GFAP serum concentrations in patients with IS, ICH, and stroke mimics.
The box boundaries mark the 25th and 75th percentile; the line within the box indicates the median. Whiskers above and below the box mark the 90th and 10th percentiles. Outliers [1.5 − 3 × interquartile range (IQR)] are marked with circles, extreme values (>3 × IQR) are marked with asterisks. The *y* axis is log transformed. Mann–Whitney test, $*P < 0.001$.

analyze the association between ICH localization and GFAP levels. A significance level of $\alpha = 0.05$ was chosen for all tests.

Results

We included 202 patients. Mean age was 73 years and 51% of patients were female. 45 patients had ICH, 146 patients had IS, and 11 patients were classified as stroke mimics. The mean time from symptom onset to hospital admission was 1 h 53 min. Table 1 depicts the baseline variables stratified according to the final diagnosis (ICH, IS, stroke mimic).

GFAP serum concentrations were significantly higher in ICH patients than in patients with IS or stroke mimics [median (interquartile range) 0.16 μg/L (0.04–3.27) vs 0.01 μg/L (0.01–0.01), $P < 0.001$, Fig. 1]. ROC analysis (Fig. 2) revealed a GFAP concentration of 0.03 μg/L to be the optimal cutoff to distinguish ICH from IS and stroke mimics [area under the curve (AUC) 0.872 (95% CI 0.802–0.942), $P < 0.001$]. At this cutoff, ICH was detected with a sensitivity of 77.8%, a specificity of 94.2%, and an overall accuracy of 90.6% (PPV: 79.5%, NPV: 93.7%). We then attempted to optimize NPV by means of reducing the cutoff to 0.015 μg/L. This led to a marginal improvement of the NPV to 93.9%. However, specificity decreased to 88.5%, and PPV decreased to 66.7%.

In patients with ICH, hemorrhage volumes and GFAP values were found significantly correlated (Spearman ρ 0.553, $P < 0.001$; Fig. 3). Table 2 shows the distribution of ICH locations, respective ICH volumes, and GFAP serum concentrations. Median lobar bleed volume was larger than deep bleed volume [53.2 mL (28.2–103.0) vs 15.2 mL (7.4–27.9); $P = 0.001$]. Accordingly, patients with lobar ICH had significantly higher median GFAP serum concentrations as compared to patients with deep ICH [1.73 μg/L (0.07–9.92) vs 0.06 μg/L (0.01–0.34); $P = 0.020$]. In the group of patients with deep ICH, the 15 patients with basal ganglia bleeds [median volume 15.2 mL (8.1–21.6)] had low serum GFAP concentrations [median 0.06 μg/L (0.01–0.34)]. The 2 patients with brainstem bleeds revealed very low GFAP concentrations [median 0.01 μg/L (0.01–0.01)]. One patient with a cerebellar hemorrhage with a volume of 59 mL had a pronounced GFAP concentration of 1260 μg/L. In a multivariate logistic regression analysis including ICH

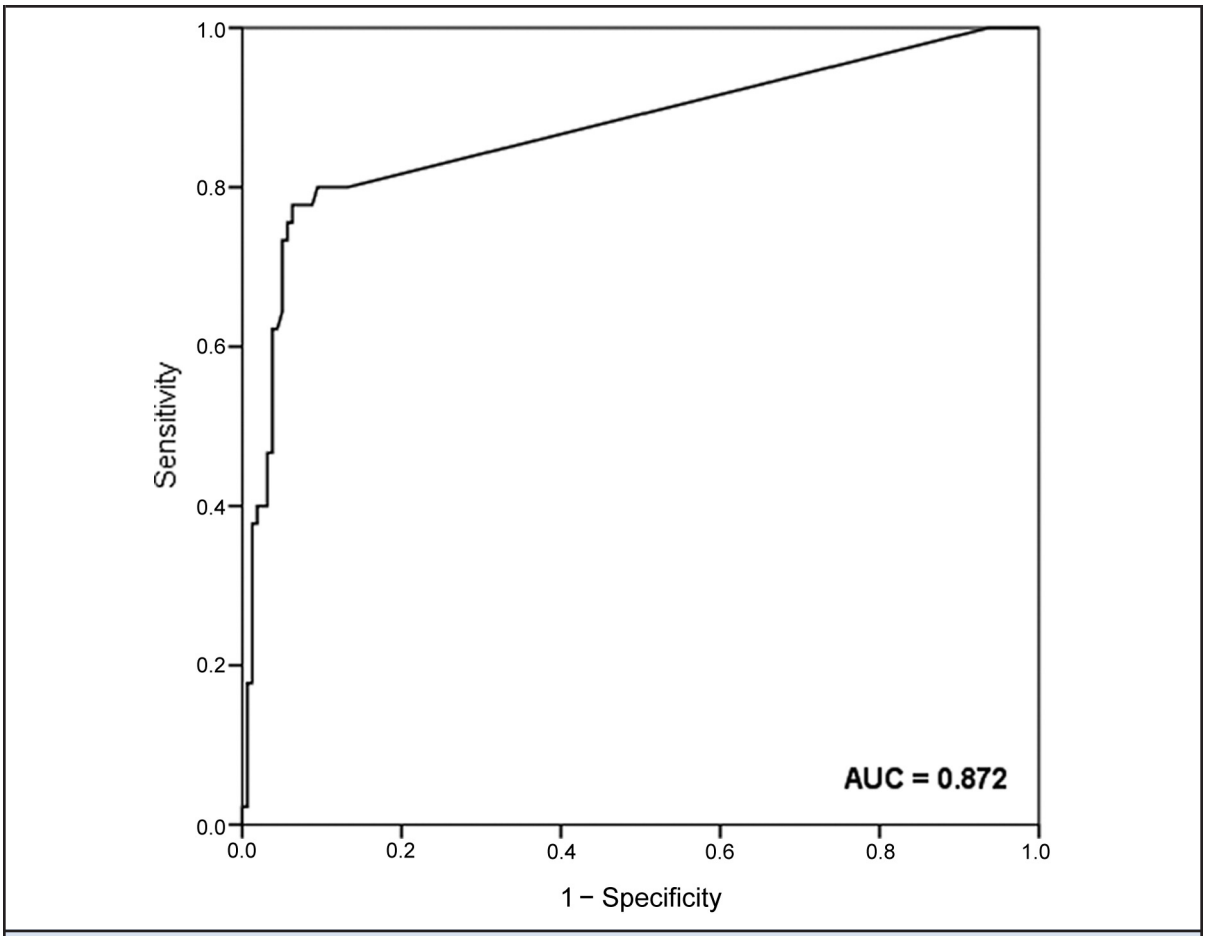

Fig. 2. ROC curve analysis depicting the diagnostic accuracy of serum GFAP for the differentiation of patients with ICH and patients with ischemic stroke (including stroke mimics).
AUC: 0.872, CI: 0.802–0.942, *$P < 0.001$.

location as the dependent variable and ICH volume and GFAP as independent variables, we found a significant association between ICH volume and lobar ICH location. An association of GFAP concentrations with lobar ICH location independent of ICH volume was not obtained.

COMBINED ANALYSIS OF BE FAST I AND BE FAST II

To analyze whether the time span elapsed since symptom onset and clinical severity have an influence on diagnostic accuracy of the GFAP test, we performed a combined analysis of the present data (BE FAST II) and the data derived from the BE FAST I study *(10)* with a total number of n = 401 patients (ICH: n = 83, IS and stroke mimics: n = 318). To exclude any influence caused by the assay modifications between the BE FAST I and BE FAST II studies, we focused on analyzing diagnostic accuracy measures for GFAP above and below the respective cutoff values only. The combined analysis revealed a sensitivity of 80.7%, a specificity of 95.0% and an overall diagnostic accuracy of 92.0% (PPV 80.7%, NPV 95.0%) to detect ICH in patients presenting with symptoms of acute stroke. Overall diagnostic accuracy was comparable between patients who were admitted to the hospital very early after symptom onset (<60 min, n = 115, 93.0%) and those admitted later (60–120 min, n = 135, 93.0%; >120 min, n = 143, 90.2%). Similarly, sensitivity and specificity values remained stable over these admission time windows (sensitivity 85.2% vs 77.3% vs 80.0%, specificity 95.5% vs 96.4% vs 92.9%). When stratifying for the clinical severity of the neurological deficit (quantified by the NIHSS score on admission) sensitivity of the GFAP test was markedly lower in clinically less severely affected patients (NIHSS <10, 66.6%) in comparison to those patients with an NIHSS ≥10 (83.0%). In contrast, specificity (97.8% vs 94.6%) and overall diagnostic ac-

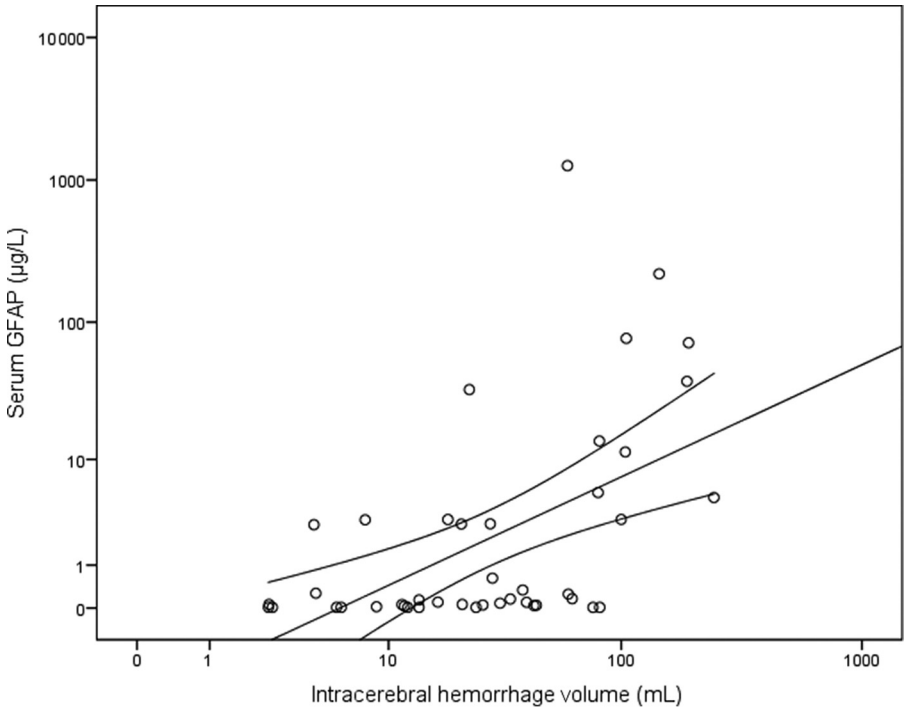

Fig. 3. Scatterplot showing the relationship between serum GFAP concentration (µg/L) and intracerebral hemorrhage volume (mL).
Regression line with 95% CI (space between the 2 curves). The *x* and *y* axes are log transformed. Spearman ρ 0.553, $^*P < 0.001$.

curacy (94.3% vs 91.8%) remained comparable in both stroke severity groups.

Discussion

To implement a diagnostic test in the clinical setting it is of paramount importance that it produces comparable results in different patient populations. This prospective multicenter study confirms in an independent cohort that GFAP serum concentrations are indicative of presence or absence of ICH in patients with acute stroke symptoms. The comparison of our data with smaller proof-of-principle investigations *(9, 21)* as well as with the results of our first prospective multicenter trial (BE

Table 2. ICH localization, volume, and corresponding serum GFAP concentration.[a]

ICH localization	Serum GFAP concentration, µg/L, median [IQR]	ICH volume, mL, median [IQR]
Deep, n = 23	0.06 [0.01–0.34]	15.15 [7.35–27.85]
Thalamus, n = 5	0.25 [0.09–1.71]	13.70 [6.15–51.49]
Basal ganglia, n = 15	0.06 [0.01–0.34]	15.15 [8.10–21.59]
Brain stem, n = 2	0.01 [0.01–0.01]	15.0 [5.70–15.00]
Cerebellar, n = 1	1260.10 [-]	59.5 [-]
Lobar, n = 20	1.73 [0.07–9.92]	53.15 [28.15–103.00]
Frontal, n = 4	3.92 [0.78–5.32]	55.42 [28.70–202.55]
Temporal, n = 3	0.06 [0.01–0.06]	76.20 [2.52–76.20]
Parietal, n = 12	1.50 [0.06–56.93]	53.15 [28.03–103.75]
Occipital, n = 1	0.6 [-]	28.6 [-]

[a] Because of missing data concerning localization, 2 patients with ICH had to be excluded.

FAST I) *(10)* shows that the diagnostic accuracy measures are very consistent between the studies. Overall, approximately 80% of patients with acute ICH were correctly diagnosed by means of a single GFAP test, and 95% of patients with IS did not have increased GFAP values (above the stated cutoff) *(22)*. In comparison with making a diagnosis only based on the pretest probability of the presence of ICH in a patient with acute stroke symptoms (which is about 10%–20% *(23)*, a positive GFAP test result will vastly increase the accuracy of diagnosing acute ICH. However, ruling out ICH by means of this test is not possible with justifiable accuracy.

Our results are consistent with previous studies regarding the robust correlation between GFAP concentrations and ICH volumes. The immediate astroglial cell destruction caused by the expanding hematoma is assumed to be responsible for the rapid GFAP release into the intravasal blood in acute ICH *(8)*. GFAP is a structural protein that is not actively secreted by cells *(18, 24)*. Apart from stroke and traumatic brain injury, glioblastoma is the only disease entity that has been found to be associated with increased GFAP concentrations in serum *(13)*. In IS, the loss of cellular integrity does not occur before 6–12 h of vessel occlusion *(8)*. Therefore in IS GFAP is released with delay, providing the basis for which we chose the up to 6-h diagnostic time window in the current study.

A critical finding of both the BE FAST I and BE FAST II study is that not all ICH patients had positive GFAP test signals (as determined by one-time-only testing in the acute phase). The fact that sensitivity did not improve in BE FAST II (78%) in comparison to BE FAST I (84%) despite the use of new antibodies with high affinity suggests that the reduced sensitivity is not a matter of testing issues only. Rather, pathophysiological factors play an important role in this context. It is conceivable that primarily intraventricular or subarachnoid hemorrhages (without initial ICH component) fail to trigger GFAP release, as in these hemorrhage types there is no immediate parenchymal (astroglial) destruction *(8, 20)*. In our cohort, intraventricular hemorrhage did not occur in an isolated manner, but was associated with parenchymal hemorrhage of large volumes in most cases. Thus, this question could not be addressed. In the present study, deep ICH location (thalamus, basal ganglia, or brain stem) was associated with lower GFAP serum concentrations compared to lobar ICH location. However, an important confounder in this analysis is the ICH volume, which was lower in patients with deep ICH than in patients with lobar ICH. In a multivariate regression model, we confirmed an association between ICH volume and lobar ICH location, but an association between GFAP concentrations with lobar ICH location independently from ICH volume was not observed. The strikingly high GFAP concentration in a patient with cerebellar ICH is remarkable. It might be hypothesized that the close proximity of this bleeding to the subarachnoid space may have led to a more pronounced release of GFAP into the systemic circulation. On the other side, the high GFAP concentration could be also explained owing to a bleeding volume of 59 mL, causing extensive tissue destruction in the *posterior fossa* of the brain. Since GFAP expression is not evenly distributed throughout the brain *(25)*, it is possible that ICH location itself influences serum GFAP concentration. The current study did not have the statistical power to detect or rule out an association between hemorrhage location and GFAP serum concentrations.

To date, a GFAP point-of-care test for stroke patients, similar to the troponin test used in the diagnostic work-up of myocardial infarction, does not exist. Nevertheless, one may speculate about potential clinical implications of prehospital GFAP testing in acute stroke patients. A reliable prehospital diagnosis of ICH would enable a quick transit of patients with a high likelihood of having ICH to hospitals with an affiliated neurosurgical department and intensive care infrastructure. Particularly in geographical areas that are underserved regarding state of the art stroke centers, such a test may help to optimize prehospital triage. Ongoing research projects, e.g., at the Norwegian Air Ambulance service, are specifically investigating the utility of GFAP-based prehospital stroke diagnostics in medically underserved areas *(26)*.

In patients diagnosed with ICH, early blood pressure management *(27)* and, if applicable, reversal of anticoagulation *(28)* have a reasonable risk/benefit ratio.

To be of benefit in patients with acute IS, a high NPV (i.e., high probability that patients with low GFAP values do not have ICH) is desirable, since this would hypothetically allow prehospital thrombolytic treatment based on a serum test. The NPV in our primary analysis was 93.7%. A further reduction of the cutoff did not increase the NPV, but led to a significant increase in patients with false positive results. Thus, ruling out ICH is not possible based on a single GFAP serum test. At present, this is a clear contraindication against the application of thrombolysis. However, even if diagnostic accuracy is not perfect, decision models have been published advocating for the initiation of a prehospital thrombolysis treatment under certain circumstances without prior brain imaging. Under the premise that a future point of care test used has 75% to 80% sensitivity to detect ICH, early thrombolysis based on serum tests may become conceivable *(7)*. A combined set of biomarkers, preferably with one biomarker indicating cerebral ischemia and one ICH, will likely increase diagnostic accuracy.

The current study has limitations that need to be addressed: First, only few patients with stroke mimics were included. Thus, conclusions concerning diagnostic

accuracy in patients having a final diagnosis different from IS or ICH are limited. However, it was already shown that in healthy controls and in many other neurological diseases GFAP serum concentrations are very low *(18, 20)*. Second, since ischemic brain damage also leads to cellular disintegration and therefore to a delayed GFAP release we had to exclude patients with previous stroke and transient ischemic attack, resulting in a selection bias in our study population *(29)*. Third, it may be interesting to know if other clinical variables (renal function, systemic inflammation, preexisting cerebral small vessel disease, hypercholesterolemia, smoking) influence GFAP serum concentrations and whether subgroups of acute stroke patients (e.g., those chosen to undergo MRI vs those undergoing CT imaging) have different GFAP concentrations. Fourth, considering the two BE FAST trials and previous explorative studies, different GFAP cutoff points have been obtained, likely owing to modifications of the GFAP assay (see methods) and lack of a reference standard *(10, 21)*. However, it has consistently been observed that cutoff points were located at or near the upper limit of the reference interval. Furthermore, in our current study GFAP serum concentrations in healthy individuals (95th percentile at 0.03 μg/L) and in patients with acute IS (median 0.01 μg/L) resembled each other. This confirms that GFAP serum concentrations in patients with acute IS in an early time window are not yet increased and thus are similar to GFAP concentrations in healthy controls. Fifth, no interference of hemolysis on test performance was observed up to at least 6 g/L of hemoglobin. However, an individual graduation of samples according to hemolysis levels was not performed.

In conclusion, serum GFAP is a reliable biomarker to identify ICH in patients presenting with symptoms suggestive of acute stroke.

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References

1. Truelsen T, Bonita R. The worldwide burden of stroke: Current status and future projections. Handbook of clinical neurology 2009;92:327–36.
2. Hemphill JC, 3rd, Greenberg SM, Anderson CS, Becker K, Bendok BR, Cushman M, et al. Guidelines for the management of spontaneous intracerebral hemorrhage: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. Stroke 2015;46:2032–60.
3. Wardlaw JM, Murray V, Berge E, del Zoppo G, Sandercock P, Lindley RL, Cohen G. Recombinant tissue plasminogen activator for acute ischaemic stroke: an updated systematic review and meta-analysis. Lancet 2012;379:2364–72.
4. Badhiwala JH, Nassiri F, Alhazzani W, Selim MH, Farrokhyar F, Spears J, et al. Endovascular thrombectomy for acute ischemic stroke: a meta-analysis. JAMA 2015; 314:1832–43.
5. Unden J, Strandberg K, Malm J, Campbell E, Rosengren L, Stenflo J, et al. Explorative investigation of biomarkers of brain damage and coagulation system activation in clinical stroke differentiation. J Neurol 2009;256: 72–7.
6. Saver JL. Time is brain–quantified. Stroke 2006;37: 263–6.
7. Lorenz MW, Lauer A, Foerch C. Quantifying the benefit of prehospital rapid treatment in acute stroke: Benchmark for future innovative clinical trials. Stroke 2015; 46:3168–76.
8. Brunkhorst R, Pfeilschifter W, Foerch C. Astroglial proteins as diagnostic markers of acute intracerebral hemorrhage-pathophysiological background and clinical findings. Transl Stroke Res 2010;1:246–51.
9. Dvorak F, Haberer I, Sitzer M, Foerch C. Characterisation of the diagnostic window of serum glial fibrillary acidic protein for the differentiation of intracerebral haemorrhage and ischaemic stroke. Cerebrovasc Dis 2009;27: 37–41.
10. Foerch C, Niessner M, Back T, Bauerle M, De Marchis GM, Ferbert A, et al. Diagnostic accuracy of plasma glial fibrillary acidic protein for differentiating intracerebral hemorrhage and cerebral ischemia in patients with symptoms of acute stroke. Clin Chem 2012;58:237–45.
11. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Clin Chem 2003;49:7–18.
12. Lei J, Gao G, Feng J, Jin Y, Wang C, Mao Q, Jiang J. Glial fibrillary acidic protein as a biomarker in severe traumatic brain injury patients: a prospective cohort study. Crit Care 2015;19:362.
13. Tichy J, Spechtmeyer S, Mittelbronn M, Hattingen E, Rieger J, Senft C, Foerch C. Prospective evaluation of serum glial fibrillary acidic protein (GFAP) as a diagnostic marker for glioblastoma. J Neurooncol 2016;126: 361–9.
14. Leitlinien für das Management der arteriellen Hypertonie. c2013. ESC Pocket Guidelines. https://www.hochdruckliga.de/tl_files/content/dhl/downloads/2014_Pocket-Leitlinien_Arterielle_Hypertonie.pdf (Accessed November 2016).
15. Diagnostik und Therapie der Dyslipidämien. c2012. ESC Pocket Guidelines. http://leitlinien.dgk.org/files/2012 Pocket-Leitlinien Dyslipidaemie.pdf (Accessed November 2016).
16. Diabetes. c2013. ESC Pocket Guidelines. http://leitlinien.dgk.org/files/2014_Korrigierte_Fassung_PLL_Diabetes_Internet.pdf (Accessed November 2016).
17. Kothari RU, Brott T, Broderick JP, Barsan WG, Sauerbeck LR, Zuccarello M, Khoury J. The ABCs of measuring intracerebral hemorrhage volumes. Stroke 1996;27: 1304–5.
18. Missler U, Wiesmann M, Wittmann G, Magerkurth O, Hagenstrom H. Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results. Clin Chem 1999;45:138–41.
19. Larsson IM, Wallin E, Kristofferzon ML, Niessner M, Zetterberg H, Rubertsson S. Post-cardiac arrest serum levels of glial fibrillary acidic protein for predicting neurological outcome. Resuscitation 2014;85: 1654–61.
20. Mayer CA, Brunkhorst R, Niessner M, Pfeilschifter W, Steinmetz H, Foerch C. Blood levels of glial fibrillary acidic protein (GFAP) in patients with neurological diseases. PLoS One 2013;8:e62101.
21. Foerch C, Curdt I, Yan B, Dvorak F, Hermans M, Berkefeld J, et al. Serum glial fibrillary acidic protein as a biomarker for intracerebral haemorrhage in patients

with acute stroke. J Neurol Neurosurg Psychiatry 2006; 77:181–4.
22. Zhang J, Zhang CH, Lin XL, Zhang Q, Wang J, Shi SL. Serum glial fibrillary acidic protein as a biomarker for differentiating intracerebral hemorrhage and ischemic stroke in patients with symptoms of acute stroke: a systematic review and meta-analysis. Neurol Sci 2013;34: 1887–92.
23. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics–2015 update: a report from the American Heart Association. Circulation 2015;131:e29–322.
24. Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). Neurochem Res 2000;25:1439–51.
25. Torres-Platas SG, Nagy C, Wakid M, Turecki G, Mechawar N. Glial fibrillary acidic protein is differentially expressed across cortical and subcortical regions in healthy brains and downregulated in the thalamus and caudate nucleus of depressed suicides. Mol Psychiatry 2016;21:509–15.
26. Ting JY. Letter to the editors: the potential role for prehospital thrombolysis and time-critical stroke transfers in the northern Norway aeromedical retrieval system; in response to: Norum J, Elsbak TM: Air ambulance services in the Arctic: a Norwegian study. Int J Emerg Med 2011, 4:1. Int J Emerg Med 2011;4:45.
27. Anderson CS, Heeley E, Huang Y, Wang J, Stapf C, Delcourt C, et al. Rapid blood-pressure lowering in patients with acute intracerebral hemorrhage. N Engl J Med 2013;368:2355–65.
28. Pollack CV, Jr., Reilly PA, Eikelboom J, Glund S, Verhamme P, Bernstein RA, et al. Idarucizumab for dabigatran reversal. N Engl J Med 2015;373:511–20.
29. Herrmann M, Vos P, Wunderlich MT, de Bruijn CH, Lamers KJ. Release of glial tissue-specific proteins after acute stroke: a comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. Stroke 2000;31:2670–7.