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Diagnostic Accuracy of Plasma Glial Fibrillary Acidic Protein for Differentiating Intracerebral Hemorrhage and Cerebral Ischemia in Patients with Symptoms of Acute Stroke

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BACKGROUND: Glial fibrillary acidic protein (GFAP) is a biomarker candidate indicative of intracerebral hemorrhage (ICH) in patients with symptoms of acute stroke. GFAP is released rapidly in the presence of expanding intracerebral bleeding, whereas a more gradual release occurs in ischemic stroke. In this study the diagnostic accuracy of plasma GFAP was determined in a prospective multicenter approach.

METHODS: Within a 1-year recruitment period, patients suspected of having acute (symptom onset <4.5 h before admission) hemispheric stroke were prospectively included into the study in 14 stroke centers in Germany and Switzerland. A blood sample was collected at admission, and plasma GFAP was measured by use of an electrochemiluminometric immunoassay. The final diagnosis, established at hospital discharge, was classified as ICH, ischemic stroke, or stroke mimic.

RESULTS: The study included 205 patients (39 ICH, 163 ischemic stroke, 3 stroke mimic). GFAP concentrations were increased in patients with ICH compared with patients with ischemic stroke [median (interquartile range) 1.91 μg/L (0.41–17.66) vs 0.08 μg/L (0.02–0.14), $P < 0.001$]. Diagnostic accuracy of GFAP for differentiating ICH from ischemic stroke and stroke mimic was high [area under the curve 0.915 (95% CI 0.847–0.982), $P < 0.001$]. A GFAP cutoff of 0.29 μg/L provided diagnostic sensitivity of 84.2% and diagnostic specificity of 96.3% for differentiating ICH from ischemic stroke and stroke mimic.

CONCLUSIONS: Plasma GFAP analysis performed within 4.5 h of symptom onset can differentiate ICH and ischemic stroke. Studies are needed to evaluate a GFAP point-of-care system that may help optimize the prehospital triage and management of patients with symptoms of acute stroke.

Brain imaging remains the gold standard for differentiating patients with ischemic stroke and intracerebral hemorrhage (ICH)[16] *(1)*. However, performing a computerized tomography or MRI scan requires hospital admittance of patients with symptoms suspicious for acute stroke. Therefore, in the prehospital setting, no diagnosis-specific measures can be applied, such as lowering of increased blood pressure in case of acute ICH or the rapid reversal of anticoagulation in the presence of warfarin-associated ICH *(2)*. Regarding thrombolysis in patients with ischemic stroke, the need for hospital admittance causes a tremendous time-to-treatment delay *(3)*. Patients cannot be treated on site or in the ambulance as for myocardial infarction *(4)*. Because of the strong relationship between the efficacy of thrombolysis and time to treatment, any measure reducing the latter is likely to be highly beneficial *(5)*.

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[16] Nonstandard abbreviations: ICH, intracerebral hemorrhage; GFAP, glial fibrillary acidic protein; NIHSS, NIH Stroke Scale; AUC, area under the curve.

For example, the prenotification of the hospital of the impending arrival of a patient with acute stroke symptoms increases the frequency of thrombolysis and shortens the time until initiation of treatment *(6)*. Thus, having a near-patient test device able to reliably differentiate between ischemic stroke and ICH in the preclinical setting would be desirable.

Efforts have been made to identify biomarkers indicative of ischemic stroke in the early phase of the disease. Typically, the investigated proteins are key representatives of the different cascades involved in the pathophysiology of cerebral ischemia, including coagulation activation, blood–brain barrier damage, and cell death. However, none of these protein biomarkers has been found to have high enough diagnostic accuracy to be of value in routine clinical practice *(7–9)*. Data on biomarkers indicative of ICH are scarce and mostly comprise proteins that are abundantly present in glial cells, including protein S100B and glial fibrillary acidic protein (GFAP) *(7, 10, 11)*. GFAP is a highly brain-specific intermediate filament protein maintaining astroglial cell structure and migration *(12)*. Under physiological conditions, GFAP is not actively secreted from cells, and the protein is typically not detectable in the plasma of healthy individuals *(13)*. Explorative studies have shown that GFAP is released rapidly in acute ICH, whereas a more delayed release can be observed in patients with ischemic stroke *(14–16)*. This delayed release reflects the more gradual occurrence of necrosis and cytolysis found histopathologically in ischemic stroke, in contrast to the immediate cell destruction in case of ICH *(17, 18)*. GFAP may therefore function as a biomarker indicating ICH in patients with symptoms suspicious for acute stroke.

We conducted a prospective multicenter study to test the diagnostic accuracy of plasma GFAP, as measured by use of a newly developed automated electrochemiluminometric immunoassay, for the differentiation between ICH and ischemic stroke.

Methods

This study, part of the BE FAST! (Biomarker for Rapid Diagnosis of Hemispheric Stroke) study, was designed according to the guidelines of the Standards for Reporting of Diagnostic Accuracy initiative *(19)*. The study protocol was approved by the ethics committee of the University Hospital of Frankfurt am Main, Germany, and by the local ethics committees of the participating hospitals. Patients or legal representatives had to give informed consent for study participation. The study was registered at http://www.clinicaltrials.org (NCT00916864).

STUDY DESIGN

This study was performed between June 1, 2009, and May 31, 2010, at 13 certified stroke centers in Germany and 1 stroke center in Switzerland. The 14 centers included 5 university hospitals.

Patients admitted with symptoms suspicious for acute stroke were prospectively screened for the presence of the following inclusion and exclusion criteria: (*a*) time window between symptom onset and hospital admission <4.5 h, (*b*) presence of a hemiparesis at hospital admission (i.e., unilateral paresis of arm and leg, Medical Research Council Scale 0–4), and (*c*) presence of at least 1 clinical sign of hemispheric involvement (aphasia, neglect, homonymous hemianopia, gaze deviation to the contralateral side of the hemiparesis, reduced level of consciousness). Patients were excluded if they had an ischemic stroke, a transient ischemic attack, or an ICH within 3 months before the index event; if they had brain trauma within the last 3 months; or if they had a diagnosis of brain tumor at any time in their medical history. After study inclusion, the following clinical variables were recorded: age, sex, NIH Stroke Scale (NIHSS) at hospital admission, time span from symptom onset to hospital admission (in minutes), history of arterial hypertension, and history of diabetes mellitus (defined according to current guidelines) *(20, 21)*. At least 1 brain scan had to be performed within 24 h of hospital admission, but the modality (computed tomography, MRI) was not specified. The final diagnosis (gold standard) was established at hospital discharge on the basis of all available clinical data, brain imaging, laboratory testing, and other examinations. Final diagnosis was categorized as (*a*) ischemic stroke including transient ischemic attack (*International Classification of Diseases, Revision 10* I63, G45), (*b*) ICH (I61), or (*c*) other (stroke mimic).

BLOOD SAMPLING

At hospital admission, 2 mL of blood was collected in a potassium EDTA Vacutainer tube (BD Company). Blood tubes were rapidly transported to the laboratory facility of the hospitals and centrifuged at 1500*g*–2000*g* for 10 min at the earliest possibility and no longer than 1 h after blood collection. Plasma samples were immediately frozen and stored below −25 °C. Blood samples were shipped on dry ice. GFAP is known to be stable in whole blood for several days at 4 °C, and freezing and thawing for up to 4 cycles does not influence GFAP concentrations *(13)*.

ICH VOLUME DETERMINATION

In ICH patients, intracerebral hematoma volume (mL) was quantified on the basis of the first available brain scan (performed within 24 h of symptom onset) by use of the $(a \times b \times c)/2$ method *(22)*. Infarct size measure-

ments in patients with ischemic stroke were not performed.

GFAP MEASUREMENTS

Quantification of plasma GFAP concentrations was performed at Roche Diagnostics, Penzberg, Germany. All scientists involved in the measurements were fully blinded to the clinical data. Plasma samples were inspected for signs of hemolysis after thawing (classified as present or absent). The Elecsys®[17] GFAP prototype test is an electrochemiluminometric immunoassay for the in vitro quantification of GFAP in human serum and plasma. In a first step, biotin- and ruthenium-labeled monoclonal GFAP antibodies were combined with 50 μL of sample and incubated for 9 min. In the second step, streptavidin-coated magnetic microparticles were added, and the mixture was incubated for 9 min. Then the reaction mixture was transferred into the measuring cell, where the beads were captured on the surface of an electrode by a magnet. Unbound label was removed by washing the measuring cell. In the last step, voltage was applied to the electrode in the presence of a tripropylamine-containing buffer, and the resulting electrochemiluminescent signal was recorded by a photomultiplier. The GFAP concentration was calculated from the read-off on the basis of a calibration curve. This calibration curve is defined by a set of 7 master calibrators. Because no acknowledged reference method is currently available, the Elecsys GFAP assay has been standardized by weighting pure human GFAP in analyte-free human serum matrix. Each measurement was performed in full calibration mode. The measuring range of the GFAP prototype assay is between 0.05 and 150 μg/L (defined by the lower detection limit and the maximum of the master curve). The lower detection limit of the assay, defined as the lowest measurable GFAP concentration that can be distinguished from zero, was calculated as the concentration lying 2 SDs above the lowest standard, and was determined to be 0.05 μg/L. Intraassay imprecision of the assay was determined using 4 individual samples run in 21 replicates in a single run and was found to be between 1.1% and 1.9%. Interassay imprecision was determined using the same samples run in duplicates in 10 individually calibrated runs and ranged between 2.7% and 4.2%. In 132 apparently healthy individuals, the mean (SD) plasma GFAP concentration was determined to be 0.07 (0.11) μg/L using this assay.

[17] Elecsys is a trademark of Roche.

STATISTICAL ANALYSIS

We compared plasma concentrations of GFAP for patients with ICH and ischemic stroke using the nonparametric Mann–Whitney *U*-test, because GFAP concentrations had a skewed distribution. We used ROC-curve analysis to calculate diagnostic accuracy of GFAP for distinguishing between ICH and ischemic stroke, including stroke mimic. Here, the area under the ROC curve (AUC) is a summary measure over criteria and cutoff point choices. Previous explorative studies indicated that the optimal GFAP cutoff point lies near the upper limit of the GFAP reference interval in healthy individuals *(13–15)*. Thus, we predefined a GFAP plasma concentration of 0.29 μg/L (the mean plus 2 SD plasma GFAP concentration in healthy controls) as the cutoff. Diagnostic accuracy measures were obtained from cross-tabulations. We performed correlation analyses (plasma GFAP, NIHSS, ICH volume) by means of the nonparametric Spearman rank test. A binary logistic regression analysis was applied to the entire dataset including patients with ICH, ischemic stroke, and stroke mimic to test whether baseline parameters (age, arterial hypertension, diabetes mellitus) independently influenced plasma GFAP concentrations. GFAP as the dependent variable was categorized according to the upper cutoff ($\leq$0.29 μg/L vs $>$0.29 μg/L).

Results

The study included 205 patients (median 13 patients per center, interquartile range 5–19). Thirty-nine patients had a final diagnosis of ICH, 163 patients ischemic stroke, and 3 stroke mimic. Table 1 displays the baseline variables of the study population stratified according to the final diagnosis. Mean (SD) time from symptom onset to hospital admission and blood sampling was 134 (64) min in ICH, 123 (65) min in ischemic stroke, and 140 (46) min in stroke mimic.

GFAP concentrations were found to be markedly increased in patients with ICH compared with patients who had ischemic stroke [median (interquartile range, minimum, maximum) 1.91 μg/L (0.41–17.66, 0.02, 236.27) vs 0.08 μg/L (0.02–0.14, 0.00, 0.97), $P <$ 0.001]. The 3 patients classified as having stroke mimic had a final diagnosis of migraine with aura, endocarditis with septic encephalopathy, and focal epilepsy. These 3 patients had a median plasma GFAP of 0.19 μg/L (range 0.16–0.21 μg/L) (Fig. 1). GFAP concentrations in samples showing signs of hemolysis (9%) did not differ from hemolysis-free specimens in either ischemic stroke or ICH patients.

Fig. 2A shows the diagnostic accuracy of a single GFAP measurement at hospital admission for the differentiation between patients with ICH and those with

Table 1. Baseline characteristics of the study population.

	Ischemic stroke	ICH	Stroke mimic	All[a]
n (%)	163 (79.5)	39 (19.0)	3 (1.5)	205 (100.0)
Mean age, years (SD)	75.3 (13.4)	70.7 (17.4)	44.3 (23.5)	73.9 (14.8)
Men, n (%)	79 (48.5)	21 (55.3)	2 (66.7)	102 (50.0)
Patients with hypertension, n (%)	121 (74.2)	22 (57.9)	0 (0.0)	143 (70.1)
Patients with diabetes, n (%)	35 (21.5)	4 (10.5)	1 (33.3)	40 (19.6)
Median NIHSS, (interquartile range)	12 (8–18)	16 (13–20)	5	14 (8–18)
Mean time from symptom onset to hospital admission, min (SD)	122.7 (65.2)	134.0 (63.7)	140.0 (45.8)	125.0 (64.4)

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

ischemic stroke, including stroke mimic [AUC 0.915 (95% CI 0.847–0.982), $P < 0.001$]. Diagnostic accuracy remained stable when we analyzd only those patients who were admitted very early (i.e., ≤60 min, n = 52, AUC 0.904) (Fig. 2B). Diagnostic accuracy was slightly lower in patients with a less severe clinical deficit (i.e., NIHSS score ≤14, n = 104, AUC 0.873) (Fig. 2C) compared with those patients having a more severe functional deficit (i.e., NIHSS score above the median score of 14, n = 97, AUC 0.944) (Fig. 2D).

When we applied the predefined cutoff of 0.29 μg/L for discriminatory analysis, the diagnostic sensitivity and specificity of plasma GFAP for the differentiation between ischemic stroke and ICH were 84.2% and 96.3%, respectively (positive and negative predictive value 84.2% and 96.3%, respectively). The diagnostic specificity of the GFAP test increased to 98.8% when 0.5 μg/L was selected as the cutoff (diagnostic sensitivity 73.7%), and to 100% when 1.0 μg/L was selected as the cutoff (diagnostic sensitivity 60.5%). A post hoc analysis revealed that the optimal cutoff of 0.28 μg/L for differentiating ICH from ischemic stroke in our dataset was nearly identical with our predefined cutoff.

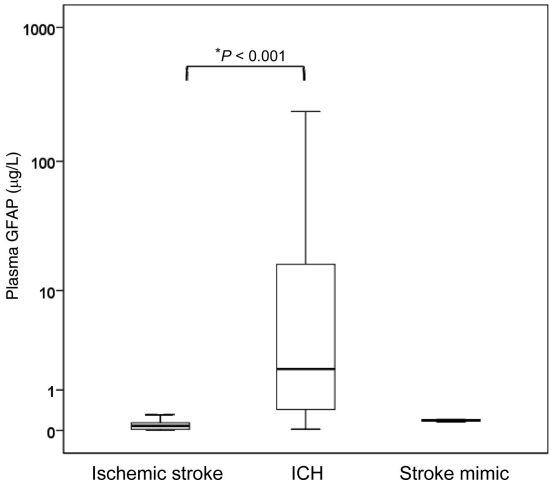

Fig. 1. Box plots illustrating the distribution of GFAP plasma concentrations in patients with ischemic stroke, ICH, and stroke mimics.

The boundaries of the box indicate the 25th and 75th percentile, and the line within the box marks the median. Whiskers above and below the box indicate the 90th and 10th percentiles. The *y* axis is log transformed.

In ICH patients, the median hematoma volume, calculated on the basis of first available brain imaging, was 39.8 mL (interquartile range 6.1–97.7 mL, minimum 2.4 mL, maximum 179.0 mL). Hematoma volume was positively correlated with plasma GFAP values ($P = 0.046$). The 2 ICH patients with the lowest hematoma volumes (2.4 and 2.7 mL) did not show an increase in GFAP. However, all 5 ICH patients with hematoma volumes between 3 and 10 mL did show positive GFAP signals (median 1.3 μg/L). A significant correlation was found between NIHSS values and GFAP plasma concentrations in ICH patients ($P = 0.022$), whereas no relationship between these 2 parameters was found in ischemic stroke patients ($P = 0.472$) (Fig. 3A and 3B).

Based on the entire dataset including patients with ICH, ischemic stroke, and stroke mimic, a binary logistic regression analysis did not reveal age, arterial hypertension, or diabetes mellitus to independently influence GFAP plasma concentrations (Table 2).

Discussion

Our study was based on a plausible pathophysiological concept. GFAP, a highly brain-specific astroglial protein, is detectable in very low concentrations in the

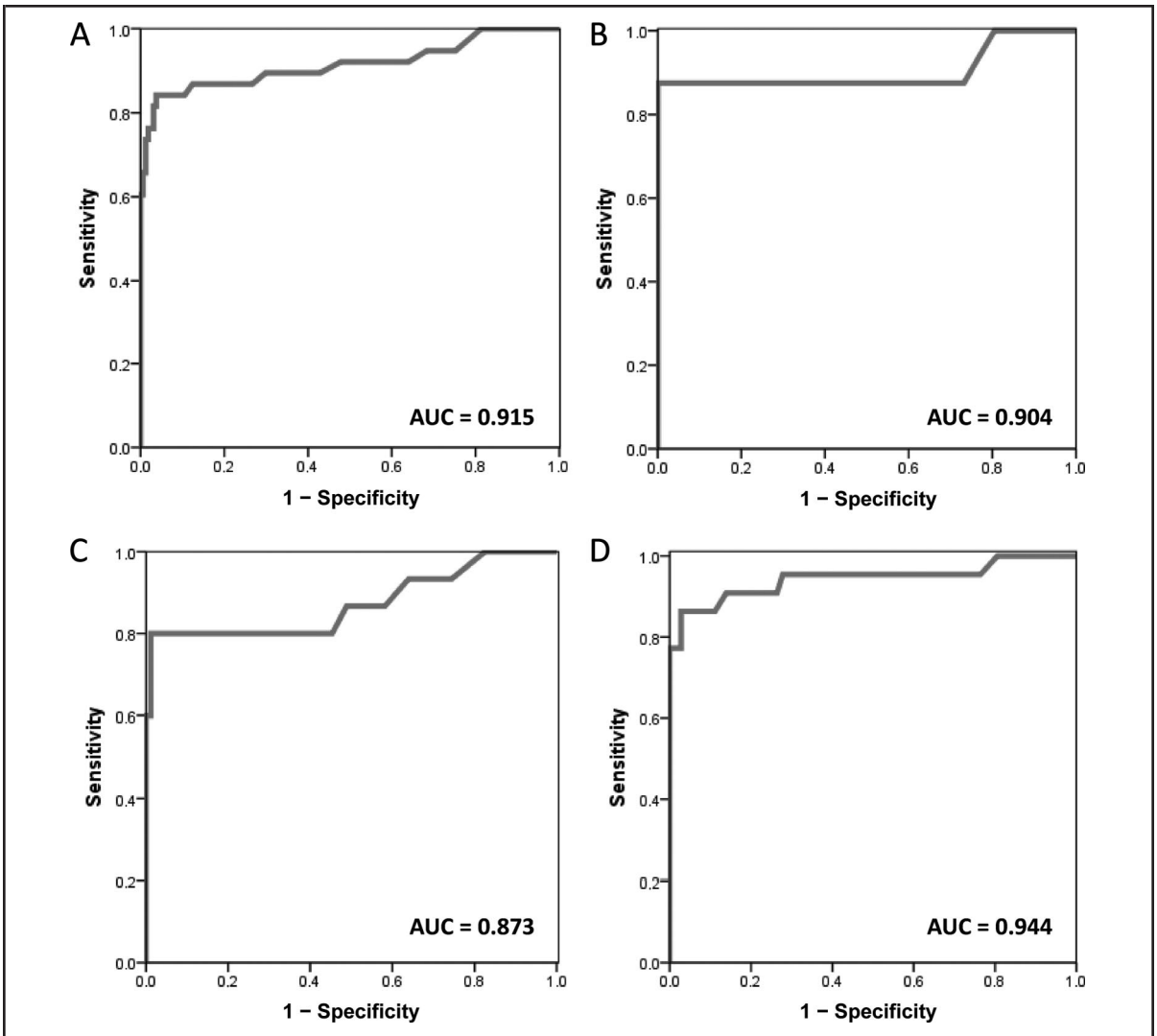

Fig. 2. ROC curve analyses for the differentiation between patients with ICH and patients with ischemic stroke (including stroke mimics).

(A), All patients (n = 205; AUC 0.915). (B), Patients admitted within the first 60 min (n = 52; AUC 0.904). (C), Patients with a moderate neurological deficit (NIHSS ≤14, n = 104; AUC 0.873). (D), Patients with a severe neurological deficit (NIHSS >14, n = 97; AUC 0.944).

blood of healthy individuals *(13)*, but it is released rapidly into plasma in the presence of an expanding intracerebral bleed, owing to the instantaneous destruction of astrocytes and the blood–brain barrier *(14, 15, 17)*. In contrast, in ischemic stroke, the structural integrity of brain cells and the blood–brain barrier are preserved for a longer time after symptom onset. Necrotic cell death and cell lysis typically do not occur within the first 6–12 h after vessel occlusion *(17, 18)*. This histopathological observation correlates well with the finding that astroglial proteins (S100B, GFAP) are released into plasma with delay, not reaching peak concentrations before 48–72 h after ischemic stroke onset *(23–26)*. A detailed analysis of the release kinetics of GFAP in ischemic stroke revealed that 75% of all values were still <0.04 μg/L 12 h after the onset of symptoms, whereas the median GFAP concentration for ICH patients was 0.56 μg/L at that time point. At 24 and 48 h, however, the median GFAP concentration in ischemic stroke was increased, and the highest GFAP values were, in absolute numbers, comparable to the GFAP concentrations (≥10 μg/L) reached at earlier time points in ICH patients *(14)*. Moreover, because our pathophysiological concept is based on differences in the

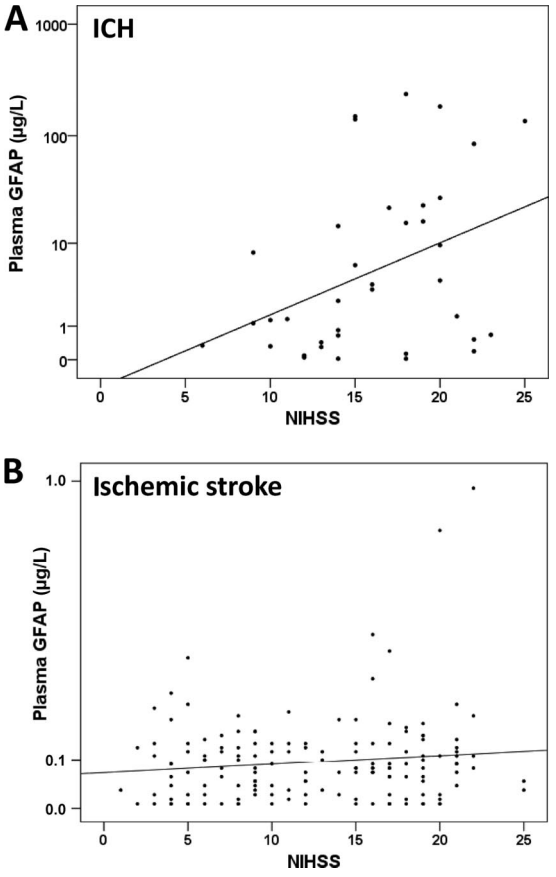

Fig. 3. Scatter plot displaying the relationship between plasma GFAP and stroke severity (assessed in terms of NIHSS values) for patients with ICH (A) ($P = 0.022$) and ischemic stroke (B) ($P = 0.472$).

Both *y* axes are log transformed.

Table 2. Results of the binary logistic regression analysis calculated on the basis of the entire dataset including patients with ICH, ischemic stroke, and stroke mimic.[a]

	Exp (B)[b]	95% CI	*P*
Age	1.002	0.977–1.028	0.863
Arterial hypertension	0.818	0.359–1.866	0.633
Diabetes mellitus	0.437	0.142–1.343	0.148

[a] GFAP as the dependent variable was categorized according to the cutoff ($\leq$0.29 μg/L vs $>$0.29 μg/L). Age, arterial hypertension, and diabetes mellitus were entered as independent variables.
[b] Exponentiation of the β coefficient.

kinetics of cellular destruction between ischemic stroke and ICH, it may not be specific for nor limited to GFAP alone. Other brain-specific proteins may follow similar release kinetics and may therefore also function as diagnostic biomarkers of ICH.

It is important to note that the time window in which high GFAP blood values indicate ICH is likely to end 6–12 h after symptom onset *(14)*. Thereafter, GFAP is detectable in ischemic stroke patients as well. As a consequence, a well-defined time point for the onset of symptoms appears to be critical for the performance of the test. We assume the diagnostic accuracy of the measure to be high within the established time window for acute stroke treatment (4.5 h) *(27, 28)*, but patients with an unknown time point for the onset of symptoms ["wake-up strokes" *(29)*] may not qualify for GFAP testing.

The strong correlation between plasma GFAP concentrations and ICH volume was well characterized in previous investigations and confirmed in this study *(15)*. Furthermore, the present study revealed a significant correlation between initial stroke severity, assessed in terms of NIHSS values, and GFAP concentrations in ICH patients because the severity of clinical symptoms depends on bleeding volume *(30)*, this supports the hypothesis that the GFAP release into plasma is tightly linked to the amount of astroglial cell destruction caused by the expanding hematoma. Conversely, we confirmed that patients with ischemic strokes do not show increased GFAP in the early phase of the disease, even if they demonstrate hemispheric stroke syndromes, a finding that suggests a critical ischemia in large parts of the affected vascular territory. As discussed above, the release of GFAP into plasma reflects astroglial cell death with loss of cellular integrity and cell lysis. However, the number of cells that will die has not yet been determined in the very early phase of ischemic stroke *(17)*. It is known that a transient perfusion deficit alone leading to functional but not structural damage on the cellular level does not cause GFAP release into the bloodstream *(26)*. For example, a patient with an occluded middle cerebral artery who undergoes successful thrombolytic treatment with rapid vessel recanalization will not show any detectable GFAP release as long as the resulting infarction is very small or nonexistent *(31)*. This delay between functional and structural breakdown explains the lack of a significant correlation between stroke severity (NIHSS values) and GFAP concentrations in patients with ischemic stroke, in contrast to the strong correlation between these 2 parameters in ICH patients.

The results of our study indicate that GFAP may function as a valid and reliable indicator of ICH in patients with symptoms suggesting acute stroke. The diagnostic sensitivity for diagnosing ICH reached 84%, a number modestly higher than in our previous studies

(14, 15). Two reasons might account for this. First, according to the inclusion criteria of our study, only patients with hemispheric stroke syndromes were considered. This criterion may have favored the inclusion of patients with rather large intracerebral hematomas. In fact, according to the analysis stratified for NIHSS values, the diagnostic performance of the GFAP test is better in patients having a more severe stroke syndrome. Second, the analytic sensitivity of the test itself may have improved, since we have moved from a hand-pipetted ELISA test to a prototype electrochemiluminometric immunoassay designed for GFAP determination on a fully automated analyzer. However, it is still not entirely clear why a small number of intracerebral hematomas did not leave a GFAP fingerprint in plasma. One reason might be the presence of a mostly intraventricular bleed, leading to less astroglial tissue destruction despite a moderate or severe clinical syndrome. Whether the site of the bleeding (cortical vs subcortical, brainstem vs supratentorial) has any influence on GFAP release must be further evaluated. Biochemical issues may also account for the reduced diagnostic sensitivity of the test. The exact half-life of GFAP in plasma and the influence of plasma/serum proteases on the stability of the protein are not yet understood and require further investigation. Furthermore, some patients may have developed autoantibodies against GFAP as a result of previous brain injury, which may interfere with the determination of plasma GFAP. More importantly, the specificity of the measure was very high. Only a few patients with ischemic strokes had slightly increased GFAP concentrations, but the large majority had concentrations equal to those of healthy individuals. No single GFAP measurement in the 163 ischemic stroke patients was higher than 1.0 μg/L, whereas >60% of the ICH patients exceeded this value.

Stratified according to the time elapsed from symptom onset to blood sampling, our analysis revealed that the performance of the GFAP test was as high in the very early phase (≤60 min after stroke onset) as in the later phase of the 4.5-h time window. In view of the potential of GFAP for diagnosing ICH in the prehospital setting (i.e., at very early time points), this observation is important. However, it stands in contrast to our previous investigation using a different GFAP test, which suggested a lower diagnostic accuracy of the GFAP test in the very early phase of the disease (<2 h) compared with the 2- to 6-h time window *(14)*.

What may be the clinical impact of our finding? GFAP indicates ICH with a high diagnostic accuracy. If a near-patient device were able to provide rapid GFAP measurements, it may allow positive identification of patients with ICH in the prehospital setting. This could speed up the triage of patients toward the optimum facility (e.g., neurosurgery, intensive care unit), but would also introduce the opportunity for prehospital treatment. At present, we consider the rapid reversal of warfarin anticoagulation using concentrated coagulation factors to be one of the most evident indications. Patients who are on effective oral anticoagulation during ICH onset develop larger hematomas compared with patients with normal coagulation and reveal a very high short-term mortality rate of approximately 50%. The rapid normalization of the coagulation system is the only measure to avoid extensive hematoma growth *(32, 33)*. If acute hemispheric stroke symptoms occur in a patient taking warfarin, a positive GFAP test, indicating ICH with a very high diagnostic accuracy, may justify the rapid reversal of anticoagulation in the outpatient setting. An even larger impact may result if currently ongoing studies investigating the effect of acute blood pressure lowering in ICH patients provide clinically significant results *(34)*. It is likely that reducing blood pressure is most effective if applied rapidly after ICH onset *(35)*. Thus, based on a positive GFAP test, blood pressure lowering may be broadly implemented in the prehospital setting. On the other hand, it is important to mention that—because of the slightly reduced diagnostic sensitivity—a GFAP blood test will not function as a sufficient measure to rule out ICH in patients with ischemic stroke who otherwise qualify for receiving thrombolytic therapy. Rather, GFAP will allow an optimized triage to specialized stroke centers, which is likely to reduce the time interval to thrombolytic therapy *(6)*.

Very little is known about the release of GFAP into the bloodstream in the presence of intracranial pathologies other than stroke. Increased plasma GFAP concentrations were found in patients with malignant gliomas and traumatic brain injury *(36, 37)*. Due to the hemispheric stroke-specific inclusion criteria (i.e., presence of both hemiparesis and at least 1 sign of hemispheric involvement), a low number of patients with stroke mimic were enrolled in our study. Taken together, the conclusions that can be drawn from our study regarding the diagnostic specificity of the measure are limited. However, the vast majority of ischemic stroke patients, stroke mimic patients, and apparently healthy controls had very low GFAP plasma concentrations, making it unlikely that plasma GFAP increases due to other conditions are a frequent finding. As another limitation, it is important to mention that our study assessed the diagnostic accuracy of the measure in hemispheric stroke patients only. In particular, our findings cannot be transferred to patients with subcortical (lacunar) strokes. In addition, future studies are needed to characterize the influence of other clinical variables on plasma GFAP concentra-

tions, such as renal function, infections, and vascular risk factors, including dyslipidemia and smoking. Finally, in future studies, it might be worthwhile to analyze whether GFAP in ischemic stroke patients increases more rapidly in case of thrombolysis-associated hemorrhagic transformation and thus may qualify as an indicator of treatment complications. Owing to the lack of follow-up brain imaging, we were not able to address this important point in the present study.

In summary, this prospective multicenter trial proved that a GFAP test performed within 4.5 h of symptom onset is a reliable tool for the differentiation between ICH and ischemic stroke. Further studies are needed to assess the diagnostic accuracy of GFAP on a point-of-care platform. GFAP measurements in the prehospital setting may have the potential to optimize the triage and management of patients with symptoms suggesting acute stroke.

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References

1. Adams HP Jr, del Zoppo G, Alberts MJ, Bhatt DL, Brass L, Furlan A, et al. Guidelines for the early management of adults with ischemic stroke. Stroke 2007;38:1655–711.
2. Broderick J, Connolly S, Feldmann E, Hanley D, Kase C, Krieger D, et al. Guidelines for the management of spontaneous intracerebral hemorrhage in adults: 2007 update. Stroke 2007;38: 2001–23.
3. Morris DL, Rosamond W, Madden K, Schultz C, Hamilton S. Prehospital and emergency department delays after acute stroke: the Genentech stroke presentation survey. Stroke 2000;31: 2585–90.
4. Morrison LJ, Verbeek PR, McDonald AC, Sawadsky BV, Cook DJ. Mortality and prehospital thrombolysis for acute myocardial infarction: a meta-analysis. JAMA 2000;283:2686–92.
5. Lees KR, Bluhmki E, von Kummer R, Brott TG, Toni D, Grotta JC, et al. Time to treatment with intravenous alteplase and outcome in stroke: An updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials. Lancet 2010;375: 1695–703.
6. Mosley I, Nicol M, Donnan G, Patrick I, Kerr F, Dewey H. The impact of ambulance practice on acute stroke care. Stroke 2007;38:2765–70.
7. Foerch C, Montaner J, Furie KL, Ning MM, Lo EH. Invited article: Searching for oracles? Blood biomarkers in acute stroke. Neurology 2009;73: 393–9.
8. Laskowitz DT, Kasner SE, Saver J, Remmel KS, Jauch EC. Clinical usefulness of a biomarker-based diagnostic test for acute stroke: the Biomarker Rapid Assessment in Ischemic Injury (BRAIN) study. Stroke 2009;40:77–85.
9. Whiteley W, Tseng MC, Sandercock P. Blood biomarkers in the diagnosis of ischemic stroke: a systematic review. Stroke 2008;39:2902–9.
10. Delgado P, Alvarez Sabin J, Santamarina E, Molina CA, Quintana M, Rosell A, Montaner J. Plasma S100B level after acute spontaneous intracerebral hemorrhage. Stroke 2006;37:2837–9.
11. Hu YY, Dong XQ, Yu WH, Zhang ZY. Change in plasma S100B level after acute spontaneous basal ganglia hemorrhage. Shock 2010;33:134–40.
12. Eng LF, Ghirnikar RS, Lee YL. Glial fibrillary acidic protein: GFAP-thirty-one years (1969–2000). Neurochem Res 2000;25:1439–51.
13. 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.
14. 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.
15. 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.
16. 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.
17. 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.
18. Persson L, Hardemark HG, Bolander HG, Hillered L, Olsson Y. Neurologic and neuropathologic outcome after middle cerebral artery occlusion in rats. Stroke 1989;20:641–5.
19. 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.
20. Leitlinien zur Behandlung der arteriellen Hypertonie. Deutsche Hochdruckliga: Deutsche Gesellschaft für Hypertonie und Prävention. 2007. http://www.hochdruckliga.de/bluthochdruck-behandlung-leitlinien.html (Accessed November 15, 2011).
21. Kerner W, Brückel J. Definition, Klassifikation und Diagnostik des Diabetes mellitus. Diabetologie 2010;5 Suppl 2:109–12.
22. 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.
23. Foerch C, Singer OC, Neumann-Haefelin T, du Mesnil de Rochemont R, Steinmetz H, Sitzer M.

Evaluation of serum S100B as a surrogate marker for long-term outcome and infarct volume in acute middle cerebral artery infarction. Arch Neurol 2005;62:1130–4.
24. 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.
25. Missler U, Wiesmann M, Friedrich C, Kaps M. S-100 protein and neuron-specific enolase concentrations in blood as indicators of infarction volume and prognosis in acute ischemic stroke. Stroke 1997;28:1956–60.
26. Wunderlich MT, Wallesch CW, Goertler M. Release of glial fibrillary acidic protein is related to the neurovascular status in acute ischemic stroke. Eur J Neurol 2006;13:1118–23.
27. Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke RT-PA Stroke Study Group. N Engl J Med 1995;333:1581–7.
28. Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008;359:1317–29.
29. Mackey J, Kleindorfer D, Sucharew H, Moomaw CJ, Kissela BM, Alwell K, et al. Population-based study of wake-up strokes. Neurology 2011;76:1662–7.
30. Huttner HB, Kohrmann M, Tognoni E, Juttler E, Richter G, Dorfler A, et al. Clinical severity predicts time to hospital admission in patients with spontaneous intracerebral hemorrhage. Cerebrovasc Dis 2008;25:533–8.
31. Foerch C, du Mesnil de Rochemont R, Singer O, Neumann-Haefelin T, Buchkremer M, Zanella FE, et al. S100B as a surrogate marker for successful clot lysis in hyperacute middle cerebral artery occlusion. J Neurol Neurosurg Psychiatry 2003;74:322–5.
32. Aguilar MI, Hart RG, Kase CS, Freeman WD, Hoeben BJ, Garcia RC, et al. Treatment of warfarin-associated intracerebral hemorrhage: literature review and expert opinion. Mayo Clin Proc 2007;82:82–92.
33. Rizos T, Jenetzky E, Herweh C, Hug A, Hacke W, Steiner T, et al. Point-of-care reversal treatment in phenprocoumon-related intracerebral hemorrhage. Ann Neurol 2010;67:788–93.
34. Anderson CS, Huang Y, Wang JG, Arima H, Neal B, Peng B, et al. Intensive blood pressure reduction in acute cerebral haemorrhage trial (INTERACT): a randomised pilot trial. Lancet Neurol 2008;7:391–9.
35. Arima H, Anderson CS, Wang JG, Huang Y, Heeley E, Neal B, et al. Lower treatment blood pressure is associated with greatest reduction in hematoma growth after acute intracerebral hemorrhage. Hypertension 2010;56:852–8.
36. Jung CS, Foerch C, Schanzer A, Heck A, Plate KH, Seifert V, et al. Serum GFAP is a diagnostic marker for glioblastoma multiforme. Brain 2007;130:3336–41.
37. Pelinka LE, Kroepfl A, Schmidhammer R, Krenn M, Buchinger W, Redl H, Raabe A. Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. J Trauma 2004;57:1006–12.