Glial fibrillary acidic protein in acute stroke: what we know and what we need to know

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Comment on: Katsanos AH, Makris K, Stefani D, *et al*. Plasma Glial Fibrillary Acidic Protein in the Differential Diagnosis of Intracerebral Hemorrhage. Stroke 2017;48:2586-8.

The differentiation between acute ischemic stroke (AIS) and intracerebral hemorrhage (ICH) remains a challenge for physicians. Effective treatments differ between both types of stroke but require therapy initiation as soon as possible after stroke onset. Therapeutic decisions—whether we start i.v. thrombolysis and deliver patients to hospitals with endovascular service in AIS or immediately lower blood pressure and reverse anticoagulation in case of ICH—depend on differentiation between these two types of stroke (1-3). The diagnosis of stroke subtypes is currently not reliable without brain imaging by computed tomography (CT) or magnetic resonance imaging (MRI) (4). Worldwide, there is a high regional variability concerning availability of CT or MRI. In some regions, not all hospitals are CT- or MRI-equipped or cannot provide emergency imaging in a 24/7 service (5). Therefore, over the last years, stroke researchers concentrated on developing a biomarker, which could be used for differentiation of ICH and AIS without scanning the brain (6). Glial fibrillary acidic protein (GFAP) was discovered and clinically tested as one of the most promising biomarkers for this purpose. GFAP is brain specific and can be measured in blood plasma with low concentrations under physiological conditions. Elevated levels are observed in other neurological diseases such as brain tumors or traumatic head injuries but these conditions are rare in an acute stroke scenario (7). An immediate destruction of glial cells in the brain, as in ICH, can lead to a release of great amounts of the protein into the bloodstream within minutes after hemorrhage. The cell destruction mechanism in ischemic stroke is different and

evolves slower, resulting in a delayed release of GFAP into bloodstream, typically around 6 hours after stroke onset when the necrotic cells start to disintegrate. This difference in GFAP kinetic between both types of stroke creates a diagnostic window (6).

In all published clinical studies, GFAP concentrations were significantly higher in patients who had ICH compared to those with AIS, when measured in a time window <12 hours of symptom onset. The largest clinical studies with highest patient numbers were BE FAST 1 and 2. With mean times from onset to blood sampling of 134 and 115 minutes, sensitivity and specificity were 84%/96% and 78%/94% respectively (8,9). The thresholds of GFAP levels for accuracy analyses in these studies were predefined with 0.29 ng/mL in BE FAST-1 and 0.03 ng/mL (optimal) in BE FAST-2. Sensitivity of GFAP for ICH diagnosis was confirmed by Xiong *et al.* in a Chinese cohort, with similar onset to sampling times and sample size (10). Sensitivity for detecting ICH was reported with 86% and specificity with 79.6%. In this study, different methods of measurement and a rather high cut-off threshold of 0.7 ng/mL were used for accuracy analyses. As the authors discussed, ethnicity may have an influence on final results.

The study by Dvorak *et al.* revealed important information concerning kinetics of GFAP in course of acute ICH (11). GFAP levels in ICH patients reached their peak between 6 and 12 hours after onset. Even with a rather small sample size, the study findings confirmed the diagnostic accuracy reported in BE FAST-1 and suggest that the optimal time window for diagnostic tests is between 2 and 6

hours. Recent developments in pre-hospital stroke treatment opened new opportunities in clinical research in an ultra-early time window, often within one hour of onset, thus bearing the potential of guiding early treatment decisions (12,13). Again, the study of our group conducted on a mobile stroke unit (MSU) confirmed the high specificity of the GFAP tests for ICH diagnosis in the prehospital time window (100% by median sampling time 43 minutes for ICH and 88 minutes for AIS). Unfortunately, the sensitivity of the test (with a 0.29 ng/mL threshold) was low (36%) for detecting ICH. These results added information on GFAP kinetics, especially in the first hour of onset. In addition, we learned that sensitivity of GFAP in differentiating between ICH and AIS is markedly lower in patients with NIHSS <10 or ICH volumes <15 mL (9,12). There is evidence that GFAP levels are positively correlated with ICH volumes and poor clinical outcome in patients suffering from ICH (9,10,12). The idea of testing multiple biomarkers in order to enhance diagnostic accuracy was followed by research groups but they were unable to achieve higher sensitivity especially in the early time window, which has the greatest potential for therapeutic decision-making (14,15). The combination of GFAP and plasmatic retinol-binding protein 4 (RBP4) showed the most promising results (16).

All of the mentioned studies had a similar limitation, namely a relative low number of patients with ICH. The number of ICH-patients varied between 18 and 45 patients per study. These studies were underpowered for more comprehensive statistical analyses like adjusting for co-morbidities or additional clinical factors, influence of location of the bleed on GFAP levels and kinetics in the very early time window. New investigations with higher numbers of ICH-patients are therefore needed.

Recently, Katsanos et al. published data obtained from larger cohort in Greece (17). In this study, the researches were able to include 270 patients, 34 with ICH diagnosis, 5 with subarachnoidal hemorrhage (SAH), 121 with AIS, 31 with stroke mimics and 79 healthy controls. Mean time to blood sampling was 168 minutes for ICH and 179 minutes for ischemic stroke, thus compared to other studies rather late but still within 6 hours of onset. The authors report higher sensitivity (91%) and specificity (97%) of the test than reported in previous studies. The optimal cut-off threshold of GFAP chosen for accuracy analysis was 0.43 ng/mL, hence higher than in other analyses. In the BE FAST studies and the pre-hospital study from our institution, optimal thresholds were 0.03 and 0.28 ng/mL. It could be speculated about two factors that might be

responsible for these discrepancies. The mean time from onset to blood sampling was the longest of all studies cited in this editorial. GFAP levels increase in course of ICH and peak approximately between 4 and 6 hours after onset, which may partially explain the difference in sensitivities despite higher threshold for accuracy analysis (10). In addition, the method of GFAP measurement used in the study could bear another possible explanation. BE FAST-1 study and pre-hospital study by Rozanski et al. conducted the measurements of GFAP levels using Roche Diagnostics prototype immunoassay (8,12). In BE FAST-2 study samples were analyzed at Roche Diagnostics and a similar prototype immunoassay modified to reach higher sensitivity was used (9). The research group of Katsanos used Cerebral Array I Biochip by Randox Laboratories Limited. There is a remarkable difference between the detection ranges of GFAP in both utilized methods; 0.18–120 ng/mL in study by Katsanos et al. versus 0.05–150 or 0.02–100 ng/mL in BE FAST studies and in our pre-hospital sampling study (8,9,12). A direct comparison of both methods, especially in lower detection ranges, seems to be difficult. Closer look at GFAP levels in patients with subarachnoid hemorrhage, even with very low numbers of patients highlights this difference; all five patients with SAH in the study of Katsanos et al. were GFAP negative while the study of Mayer et al. showed that 5 of 9 patients had detectable levels of GFAP and mean levels were 0.13 ng/mL, below the detection limit used by colleagues from Greece (7). This difference depends probably on measurements methods applied by both groups. In knowledge of previous data, the higher sensitivity of measurements seems to be important as in the example of SAH or in time windows below 1 hour (7,12).

Yet another result of this study should be discussed. The authors found an association between GFAP levels and time of blood sampling after onset. Scatter plots and corresponding fractional polynomial lines were used and described as an inverted U-shaped curve with a peak of GFAP levels in ICH patients at 2 hours after onset. Considering findings in other studies, these results should be interpreted with caution (9-12). The authors neither assessed ICH volumes nor analysed blood samples sequentially to capture the kinetic of GFAP release into the bloodstream. Furthermore, the majority of patients were included between 2 and 3 hours after time of sampling. Existing literature suggests that GFAP levels and the kinetic of release depend on ICH volume and location. No such information was provided and thus, the result could simply be a selection bias.

Ideally, point-of-care laboratory GFAP-measurement in the blood should provide a reliable diagnosis of ICH even in a very early time window—optimally within 1 hour of onset to enable an immediate start of treatment before transport to the hospital. In regions where CT is not available within the time window for i.v. thrombolysis, biomarkers such as GFAP alone or in combination with other biomarkers could allow start of thrombolysis or at least ICH therapy in carefully selected cases.

In light of these goals, the results of Katsanos *et al.* study enforce researchers on stroke biomarkers to draw important conclusions: (I) there is a need of a reference method that offers comparative analyses of studies and meta-analyses; (II) more sophisticated analyses of GFAP levels and accuracy in shorter (15, 30 minutes) intervals, preferably within earlier time windows (<1 hour) and small ICH volumes should be applied in order to gain the information of GFAP release dynamics; (III) higher numbers of patients should be included to analyse associations between ICH location and GFAP levels; (IV) GFAP levels in SAH or subdural hematoma need to be evaluated because these hemorrhages are important and absolute contraindications for i.v. thrombolysis; (V) a reasonable combination of different biomarkers and clinical parameters would be desirable to enhance sensitivity and may improve the diagnostic value of GFAP in of acute stroke diagnosis.

If future studies can address these issues and satisfactorily answer the open questions, the use of biomarkers may indeed improve acute stroke treatment in different health systems worldwide.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Han Deng (Gastroenterology and Hepatology, Yuebei People's Hospital, Shaoguan, China).

Conflicts of Interest: Both authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/amj.2018.01.04). Heinrich J. Audebert received speaker honoraria from Boehringer Ingelheim (BI, manufacturer of alteplase; not involved in any form in the trial) and speaker honoraria as well as honoraria for consultancy from Lundbeck Pharma (sponsor of trials with desmoteplase in stroke). Dr. Rozanski has no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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