Blood biomarkers for Alzheimer's disease and related disorders

Henrik Zetterberg[a,b,c,d,e]* & Jonathan M. Schott[d,f]

[a]*Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*

[b]*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*

[c]*Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, UK*

[d]*UK Dementia Research Institute at UCL, London, UK*

[e]*Hong Kong Center for Neurodegenerative Diseases, Clear Water Bay, Hong Kong, China*

[f]*Dementia Research Centre, UCL Queen Square Institute of Neurology, University College London, London, UK*

**Correspondence:*

Henrik Zetterberg, MD, PhD

Clinical Neurochemistry Laboratory

Sahlgrenska University Hospital

S-431 80 Mölndal

Tel: +46 31 3430025

Fax: +46 31 419289

E-mail: henrik.zetterberg@clinchem.gu.se

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disease, and the single commonest cause of dementia. Many other diseases can, however, cause dementia and differential diagnosis can be challenging especially in early disease stages. For most neurodegenerative dementias, accumulation of brain pathologies starts many years before clinical onset; the ability to detect these pathologies paves the way for targeted disease-modifying prevention trials. AD is associated with β-amyloid and tau pathologies which can be quantified using cerebrospinal fluid and imaging biomarkers and, more recently, using highly sensitive blood tests. While for the most part specific biomarkers of non-AD neurodegenerative dementias are lacking, non-specific biomarkers of neurodegeneration are available. This review summarizes recent advances in the neurodegenerative dementia blood biomarker research, and discusses the next steps required for clinical implementation.

Background

Alzheimer's disease (AD) is a progressive neurodegenerative dementia for which disease-modifying treatments, likely to be the most effective in early disease stages (or even in primary prevention settings), are now being developed at a rapid pace. AD-related pathologies, key amongst which are extracellular amyloid β plaques, intra-neuronal tau tangles and neurodegeneration, are evident in the brain decades before symptom onset; it is increasingly recognised that a pre-symptomatic phase whereby pathologies accumulate years before symptoms is a common feature of most neurodegenerative diseases. As the field moves to treating ever earlier making a diagnosis on clinical grounds becomes ever more difficult; and, by definition, diagnostic criteria that rely on cognitive impairment preclude diagnosis in the pre-clinical phase. While genetics plays a significant part in the development of AD and polygenic risk scores are emerging as a means of determining an individual's risk of

developing AD,[1] dynamic biomarkers which can determine the onset, profile, and intensity of neurodegeneration-related brain changes in individual patients, independent of genetic background, are required for diagnosis, prognosis, and for clinical trials – both as inclusion and outcome measures. In parallel with the development of novel treatments, intense research over many years means that we now have fluid and imaging biomarkers that may serve these purposes. While reliable cerebrospinal fluid (CSF) and imaging biomarkers for AD pathologies and neurodegeneration have been available for some time, the field has been galvanised in recent years by the development of blood-based biomarkers. This overview paper summarises recent progress in the development of these blood tests, their strengths and limitations, and some thoughts about how they could best be utilised in clinical trials and clinical practice.

Blood biomarkers for amyloid β pathology

CSF Aβ42/Aβ40 is a robust biomarker for cerebral Aβ pathology. Numerous clinical and research studies confirm that this biomarker has a clear bimodal distribution, with a relatively small overlap (grey zone) of results close to the cut-point for positivity. A low Aβ42/Aβ40 ratio is seen in AD, reflecting selective reduction of Aβ42 in the CSF likely due to deposition in amyloid plaques within the brain.[2] Recent technological developments now mean than similar reductions in Aβ42/Aβ40 can be quantified in plasma.[3] Several immunoprecipitation mass spectrometry (IP-MS) methods have been developed to extract Aβ from plasma, which is then subjected to MS-based quantification.[4-6] Using such methods, clear group-level reductions in plasma Aβ42/Aβ40 levels are observed in those with positive amyloid PET scans, *i.e.*, those with evidence of cortical fibrillar Aβ deposition, compared with amyloid PET-negative individuals.[7] A range of immunochemical tests for plasma Aβ42/Aβ40 have been developed, which are easier than MS methodologies to implement in routine clinical

chemistry laboratory settings.[8] However, in a head-to-head comparison, IP-MS methods significantly outperformed immunoassay-based techniques for detecting Aβ pathology, with areas under the curve (AUCs) ranging from between 0.69-0.78 for the different immunoassays compared with 0.86 for the best performing IP-MS method.[7] Importantly, and as is the case in CSF, plasma Aβ42/Aβ40 reduction is consistently seen in cognitively normal Aβ-positive individuals to a similar extent as seen in patients with cognitive impairment.[8] This paves the way for blood testing to be used to identify Aβ-positive individuals for clinical trials in the preclinical phase of AD; and in due course perhaps even for population-based screening.

However, a major problem with using plasma Aβ42/Aβ40 as an index of Aβ pathology is the small difference between Aβ-positive and -negative individuals. Aβ42/Aβ40 is reduced only of the order of 8-15% in plasma compared with 40-60% in CSF; and most amyloid-positive individuals' plasma values lie close to the positive/negative cut-off.[9] As opposed to CSF measures which are already in wide use, the reduced robustness of the plasma assays presents a significant challenge for these tests to be implemented in routine clinical practice – even small drifts in assay performance over time risk individuals being misclassified. Finding ways to improve the robustness of plasma Aβ tests that can be easily implemented in clinical laboratory practice is an important research topic.

Blood biomarkers for tau pathophysiology

CSF phosphorylated tau (P-tau) is an established biomarker for tau pathophysiology in AD.[10] Several research groups have now developed very sensitive blood-based P-tau assays for use as blood biomarkers of AD-related tau pathology. These include assays for tau phosphorylation at various sites including at amino acids 181 (P-tau181), 217 (P-tau217) and

231 (P-tau231).[11] Neuropathology studies have shown that plasma P-tau concentration is related to both the density of Aβ plaques and tau tangles, and that concentrations of all of these different plasma P-tau variants can differentiate cases with significant AD brain pathology from those without.[12-14] Importantly, increased plasma P-tau concentration seems to be specific to AD and is not seen in other tauopathies including primary age-related tauopathy (PART), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) or Pick's disease.[12-15] In several large-scale clinic-based studies, plasma P-tau has been shown to accurately separate AD dementia from other neurodegenerative diseases with high diagnostic accuracy.[16] Plasma P-tau levels are increased 2.5-6-fold in AD dementia compared with non-AD disorders. While all the P-tau moieties perform well, the largest relative increases in the symptomatic stages of the disease seem to be observed with P-tau217.[13,17] It is more unclear how well different plasma P-tau variants preform in the pre-symptomatic phase of AD, but evidence suggests that plasma P-tau217 and P-tau231 become abnormal shortly after Aβ-PET becomes abnormal.[12,18] As opposed to plasma Aβ42/40 where MS methods outperform immunochemical assays, all of the plasma P-tau isoforms mentioned above can be measured reliably using immunochemical methods, with a number of different research and commercial platforms now becoming available. While this bodes well for clinical implementation, head-to-head comparisons suggest that some commonly used assays have lower performance than others.[11,19] There remains considerable work to be done before plasma P-tau can be widely implemented as a clinical test, including not only choice of assay platform but also efforts to standardise methods between labs, ensure longitudinal stability, and determine appropriate clinical cut-points.

Plasma P-tau levels increase gradually over time as AD develops, perhaps relating to the number of AD-affected neurons that still manage to synthesize and secrete tau. In particular,

P-tau217 shows increases during both the preclinical and prodromal (mild cognitive impairment) stages of the disease.[20] As well as potential uses as a diagnostic and screening tool, another potential use of plasma P-tau is as a measure of tau pathophysiology in clinical trials, including those targeting Aβ pathologies. At AAIC 2021, Lilly reported reduction in plasma P-tau217 concentration in response to donanemab treatment (unpublished results), and during the Clinical Trials on Alzheimer's Disease (CTAD) conference in November 2021, similar results (in this case P-tau181 reduction) were presented for aducanumab (unpublished results). Whilst not yet conclusive, these results have been interpreted as showing that removal of Aβ has downstream effect on tau deposition, with implications for disease modification.

Blood biomarkers for neurodegeneration

For many years, CSF neurofilament light (NfL) has been used as a neuroaxonal injury marker, elevated in many different neurological disorders including a wide range of different neurodegenerative disorders.[21] The highest NfL concentrations in CSF are seen in amyotrophic lateral sclerosis, frontotemporal dementia, atypical parkinsonian disorders, and HIV-associated neurocognitive dysfunction,[21] but NfL is also increased in AD. As well as in the CSF, NfL can be reliably measured using sensitive assays developed in the last few years in plasma (or serum); CSF and blood measures of NfL show very close correlation, and virtually all CSF findings have now been replicated in blood,[22] and this extends into the preclinical phases of many diseases. Thus, both familial AD and familial FTD mutation carriers show quite striking increases in blood NfL concentration around a decade before expected clinical onset, probably marking the onset of neurodegeneration; furthermore, the higher the increase, the more rapid clinical disease progression.[23-27] Similar results have been

observed in Huntington's disease.[28-30] And in established sporadic AD, there is a clear association of increased plasma NfL concentration with Aβ and tau positivity.[31]

When it comes to measurement in routine clinical settings plasma NfL has many advantages, including being relatively unaffected by pre-analytical factors that influence many other assay.[32-34] Centrifugation can be delayed for at least two days, and NfL can be measured from dried blood spots making NfL feasible in a wide range of remote settings.[35] NfL results may however be less easy to interpret in older age groups and especially in people older than 70 years of age, due to age-related increase in plasma NfL.[36] These age-related changes are an important challenge when considering how to use the test clinically, resulting in the development of age-specific cut-points. It is also relevant that a number of common pathologies can elevate NfL, including mild traumatic brain injury,[37] cerebrovascular insults,[38] peripheral neuropathy,[39] and kidney dysfunction,[40] all of which may need to be taken into account.

Blood biomarkers for astrocytic activation

Glial activation appears to be a rapid response to the presence of Aβ pathology. Blood-based markers of brain glial pathology have proven challenging to develop due to high extra-cerebral expression of many of the proteins, *e.g.*, in macrophages, making the blood tests less reflective of brain changes. One biomarker, glial fibrillary acidic protein (GFAP), has however shown promise in this context. The highest levels of GFAP expression is seen in brain astrocytes, and its blood concentration therefore mainly reflects astrocytic activation, and in the context of AD, an astrocytic response to Aβ accumulation in the brain.[41,42] Interestingly, the relationship with brain Aβ pathology appears stronger for plasma as opposed to CSF GFAP; and plasma GFAP is not associated with fibrillar tau pathology when

adjusting for Aβ.[42] It is currently unknown to what extent different forms of Aβ deposits activate astrocytes, and there are so far no published studies on the kinetics of this response in terms blood GFAP increase. Although GFAP is likely not AD-specific, the magnitude of change in non-AD neurodegenerative diseases, such as FTD, is relatively small compared with AD;[43] mild traumatic brain injury and cerebrovascular insults are however important potential confounders.[44,45]

Conclusion

Rapid recent developments in instrument sensitivity combined with novel assay development now mean that it is possible to detect Aβ and tau pathology in blood with high diagnostic accuracy throughout the AD continuum, from pre-clinical through to established AD dementia. Plasma Aβ42/Aβ40 measures show high diagnostic performance, but the small fold change between Aβ-positive and -negative individuals, large overlap in results between the groups, and more complex assay platforms required for optimal results (with MS outperforming immunoassays) are likely to prove challenging for widespread implementation. Plasma P-tau assays detect both Aβ pathology and tau changes and are more robust than plasma Aβ42/Aβ40, showing greater fold change between patients with AD pathology and controls and are immunoassay-based and so likely to be easier to implement widely. A potential limitation of P-tau is that elevation may occur a little later in response to amyloid accumulation than with Aβ42/Aβ40; however, robust changes are still seen prior to symptom onset, and this is not likely to be a problem unless it is imperative to detect the very earliest stage of Aβ accumulation. P-tau may also be a useful outcome measure for clinical trials of drugs targeting AD-related pathologies. For most other neurodegenerative dementias, we lack disease-specific biomarkers, but plasma NfL is a robust biomarker of axonal degeneration and is already finding utility in clinical practice and to monitor intensity of neurodegeneration

intensity in clinical trials of novel disease-modifying drugs. While much work remains in terms of assay optimisation and standardisation, blood tests show considerable promise either before, or in due course perhaps in place of, more complex and expensive CSF and imaging examinations to detect AD pathology. The ease of sampling blood also raises the potential for testing in primary care, with appropriate caveats about patient selection, interpretation of results and diagnostic pathways, which are likely to still require the involvement of specialists and, for now at least, confirmation with other tests. As with any biomarker, interpretation of blood-based biomarkers should be made in the correct clinical context; while there are moves in some research settings to define AD as a pure biomarker construct, in clinical practice they should be used as part of a diagnostic process to aid diagnosis in patients with cognitive symptoms. Whilst blood biomarkers are being evaluated and used to pre-screen for clinical trials to enrich for individuals who are likely to have neurodegenerative disease, there is currently no role for their use in screening outside of clinical trials.

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Conflicts of interest

HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. JMS has received research funding and PET tracer from AVID Radiopharmaceuticals (a wholly owned subsidiary of Eli Lilly); has consulted for Roche, Eli Lilly, Biogen, Merck and GE; received royalties from Oxford University Press and Henry Stewart Talks; given education lectures sponsored by Eli Lilly, Biogen and GE; and served on a Data Safety Monitoring Committee for Axon Neuroscience SE. He is Chief Medical Officer for Alzheimer's Research UK, and Medical Advisor to UK Dementia Research Institute.

References

1. Zhou X, Li YYT, Fu AKY, Ip NY. Polygenic Score Models for Alzheimer's Disease: From Research to Clinical Applications. *Front Neurosci* 2021; **15**: 650220.
2. Hansson O, Lehmann S, Otto M, Zetterberg H, Lewczuk P. Advantages and disadvantages of the use of the CSF Amyloid beta (Abeta) 42/40 ratio in the diagnosis of Alzheimer's Disease. *Alzheimers Res Ther* 2019; **11**(1): 34.
3. Budelier MM, Bateman RJ. Biomarkers of Alzheimer Disease. *J Appl Lab Med* 2020; **5**(1): 194-208.
4. Keshavan A, Pannee J, Karikari TK, et al. Population-based blood screening for preclinical Alzheimer's disease in a British birth cohort at age 70. *Brain* 2021; **144**(2): 434-49.

5. Nakamura A, Kaneko N, Villemagne VL, et al. High performance plasma amyloid-beta biomarkers for Alzheimer's disease. *Nature* 2018; **554**(7691): 249-54.
6. Ovod V, Ramsey KN, Mawuenyega KG, et al. Amyloid beta concentrations and stable isotope labeling kinetics of human plasma specific to central nervous system amyloidosis. *Alzheimers Dement* 2017; **13**(8): 841-9.
7. Janelidze S, Teunissen CE, Zetterberg H, et al. Head-to-Head Comparison of 8 Plasma Amyloid-beta 42/40 Assays in Alzheimer Disease. *JAMA Neurol* 2021; **78**(11): 1375-82.
8. Palmqvist S, Janelidze S, Stomrud E, et al. Performance of Fully Automated Plasma Assays as Screening Tests for Alzheimer Disease-Related beta-Amyloid Status. *JAMA Neurol* 2019.
9. Schindler SE, Bollinger JG, Ovod V, et al. High-precision plasma beta-amyloid 42/40 predicts current and future brain amyloidosis. *Neurology* 2019; **93**(17): e1647-e59.
10. Alawode DOT, Heslegrave AJ, Ashton NJ, et al. Transitioning from cerebrospinal fluid to blood tests to facilitate diagnosis and disease monitoring in Alzheimer's disease. *J Intern Med* 2021.
11. Bayoumy S, Verberk IMW, den Dulk B, et al. Clinical and analytical comparison of six Simoa assays for plasma P-tau isoforms P-tau181, P-tau217, and P-tau231. *Alzheimers Res Ther* 2021; **13**(1): 198.
12. Ashton NJ, Pascoal TA, Karikari TK, et al. Plasma p-tau231: a new biomarker for incipient Alzheimer's disease pathology. *Acta Neuropathol* 2021; **141**(5): 709-24.
13. Palmqvist S, Janelidze S, Quiroz YT, et al. Discriminative Accuracy of Plasma Phospho-tau217 for Alzheimer Disease vs Other Neurodegenerative Disorders. *JAMA* 2020; **324**(8): 772-81.
14. Lantero Rodriguez J, Karikari TK, Suarez-Calvet M, et al. Plasma p-tau181 accurately predicts Alzheimer's disease pathology at least 8 years prior to post-mortem and improves the clinical characterisation of cognitive decline. *Acta Neuropathol* 2020; **140**(3): 267-78.
15. Wennstrom M, Janelidze S, Nilsson KPR, et al. Cellular localization of p-tau217 in brain and its association with p-tau217 plasma levels. *Acta Neuropathol Commun* 2022; **10**(1): 3.
16. Leuzy A, Mattsson-Carlgren N, Palmqvist S, Janelidze S, Dage JL, Hansson O. Blood-based biomarkers for Alzheimer's disease. *EMBO Mol Med* 2022; **14**(1): e14408.
17. Karikari TK, Pascoal TA, Ashton NJ, et al. Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts. *Lancet Neurol* 2020; **19**(5): 422-33.
18. Moscoso A, Grothe MJ, Ashton NJ, et al. Time course of phosphorylated-tau181 in blood across the Alzheimer's disease spectrum. *Brain* 2020.
19. Mielke MM, Frank RD, Dage JL, et al. Comparison of Plasma Phosphorylated Tau Species With Amyloid and Tau Positron Emission Tomography, Neurodegeneration, Vascular Pathology, and Cognitive Outcomes. *JAMA Neurol* 2021; **78**(9): 1108-17.
20. Mattsson-Carlgren N, Janelidze S, Palmqvist S, et al. Longitudinal plasma p-tau217 is increased in early stages of Alzheimer's disease. *Brain* 2020; **143**(11): 3234-41.
21. Bridel C, van Wieringen WN, Zetterberg H, et al. Diagnostic Value of Cerebrospinal Fluid Neurofilament Light Protein in Neurology: A Systematic Review and Meta-analysis. *JAMA Neurol* 2019.
22. Khalil M, Teunissen CE, Otto M, et al. Neurofilaments as biomarkers in neurological disorders. *Nat Rev Neurol* 2018; **14**(10): 577-89.

23. Preische O, Schultz SA, Apel A, et al. Serum neurofilament dynamics predicts neurodegeneration and clinical progression in presymptomatic Alzheimer's disease. *Nat Med* 2019; **25**(2): 277-83.
24. Weston PSJ, Poole T, O'Connor A, et al. Longitudinal measurement of serum neurofilament light in presymptomatic familial Alzheimer's disease. *Alzheimers Res Ther* 2019; **11**(1): 19.
25. Weston PSJ, Poole T, Ryan NS, et al. Serum neurofilament light in familial Alzheimer disease: A marker of early neurodegeneration. *Neurology* 2017; **89**(21): 2167-75.
26. Illan-Gala I, Lleo A, Karydas A, et al. Plasma tau and neurofilament light in frontotemporal lobar degeneration and Alzheimer's disease. *Neurology* 2020.
27. Rohrer JD, Woollacott IO, Dick KM, et al. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. *Neurology* 2016; **87**(13): 1329-36.
28. Byrne LM, Rodrigues FB, Blennow K, et al. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. *Lancet Neurol* 2017; **16**(8): 601-9.
29. Byrne LM, Rodrigues FB, Johnson EB, et al. Evaluation of mutant huntingtin and neurofilament proteins as potential markers in Huntington's disease. *Sci Transl Med* 2018; **10**(458).
30. Rodrigues FB, Byrne LM, Tortelli R, et al. Mutant huntingtin and neurofilament light have distinct longitudinal dynamics in Huntington's disease. *Sci Transl Med* 2020; **12**(574).
31. Mattsson N, Andreasson U, Zetterberg H, Blennow K, Alzheimer's Disease Neuroimaging I. Association of Plasma Neurofilament Light With Neurodegeneration in Patients With Alzheimer Disease. *JAMA Neurol* 2017; **74**(5): 557-66.
32. Keshavan A, Heslegrave A, Zetterberg H, Schott JM. Stability of blood-based biomarkers of Alzheimer's disease over multiple freeze-thaw cycles. *Alzheimers Dement (Amst)* 2018; **10**: 448-51.
33. Verberk IMW, Misdorp EO, Koelewijn J, et al. Characterization of pre-analytical sample handling effects on a panel of Alzheimer's disease-related blood-based biomarkers: Results from the Standardization of Alzheimer's Blood Biomarkers (SABB) working group. *Alzheimers Dement* 2021.
34. Ashton NJ, Suarez-Calvet M, Karikari TK, et al. Effects of pre-analytical procedures on blood biomarkers for Alzheimer's pathophysiology, glial activation, and neurodegeneration. *Alzheimers Dement (Amst)* 2021; **13**(1): e12168.
35. Simren J, Ashton NJ, Blennow K, Zetterberg H. Blood neurofilament light in remote settings: Alternative protocols to support sample collection in challenging pre-analytical conditions. *Alzheimers Dement (Amst)* 2021; **13**(1): e12145.
36. Hviid CVB, Knudsen CS, Parkner T. Reference interval and preanalytical properties of serum neurofilament light chain in Scandinavian adults. *Scand J Clin Lab Invest* 2020; **80**(4): 291-5.
37. Karantali E, Kazis D, McKenna J, Chatzikonstantinou S, Petridis F, Mavroudis I. Neurofilament light chain in patients with a concussion or head impacts: a systematic review and meta-analysis. *Eur J Trauma Emerg Surg* 2021.
38. Pedersen A, Stanne TM, Nilsson S, et al. Circulating neurofilament light in ischemic stroke: temporal profile and outcome prediction. *J Neurol* 2019; **266**(11): 2796-806.
39. Rossor AM, Kapoor M, Wellington H, et al. A longitudinal and cross-sectional study of plasma neurofilament light chain concentration in Charcot-Marie-Tooth disease. *J Peripher Nerv Syst* 2021.

40. Koini M, Pirpamer L, Hofer E, et al. Factors influencing serum neurofilament light chain levels in normal aging. *Aging (Albany NY)* 2021; **13**(24): 25729-38.
41. Benedet AL, Mila-Aloma M, Vrillon A, et al. Differences Between Plasma and Cerebrospinal Fluid Glial Fibrillary Acidic Protein Levels Across the Alzheimer Disease Continuum. *JAMA Neurol* 2021.
42. Pereira JB, Janelidze S, Smith R, et al. Plasma GFAP is an early marker of amyloid-beta but not tau pathology in Alzheimer's disease. *Brain* 2021.
43. Heller C, Foiani MS, Moore K, et al. Plasma glial fibrillary acidic protein is raised in progranulin-associated frontotemporal dementia. *J Neurol Neurosurg Psychiatry* 2020; **91**(3): 263-70.
44. Laverse E, Guo T, Zimmerman K, et al. Plasma glial fibrillary acidic protein and neurofilament light chain, but not tau, are biomarkers of sports-related mild traumatic brain injury. *Brain Commun* 2020; **2**(2): fcaa137.
45. Mattila OS, Ashton NJ, Blennow K, et al. Ultra-Early Differential Diagnosis of Acute Cerebral Ischemia and Hemorrhagic Stroke by Measuring the Prehospital Release Rate of GFAP. *Clin Chem* 2021; **67**(10): 1361-72.