

COMMENTS AND OPINIONS

Implementing Blood Biomarkers in Stroke Research and Clinical Practice

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ABSTRACT: Blood-based biomarkers reflecting the severity of brain injury showed manifold promising applications in the management of patients with stroke. To date, NfL (neurofilament light chain) and GFAP (glial fibrillary acidic protein) represent the markers with the most support from the literature, but novel biomarker candidates are emerging. In this commentary, we discuss the potential benefits that blood biomarkers would have as additional tools for physicians and stroke specialists in the assessment of stroke risk in the general population, in the acute and postacute phase of stroke management as well as during the longitudinal monitoring of patients during rehabilitation. Moreover, we present an overview of the current applications of blood biomarkers in ongoing clinical trials and debate the still unmet needs of biomarker research in stroke that future studies should target to ease their implementation in the routine care of patients.

Key Words: biomarker ■ glial fibrillary acidic protein ■ neurofilaments ■ synucleins ■ tau protein

Most blood biomarkers under investigation in the stroke field reflect cardiac, inflammatory, and metabolic diseases, and their lack of specificity limits their use for assessing the severity of brain damage.¹ Novel biomarker candidates have been preliminarily assessed, with NfL (neurofilament light chain) and GFAP (glial fibrillary acidic protein) as biomarkers of neuroaxonal injury and astroglial activation, respectively, showing the most promising results to date.^{2,3} Markers for other pathophysiological mechanisms may have even greater potential but still need extensive experimental evaluation. These include other neuronal proteins, such as tau and its peptides, glial proteins, for example, S100B (S100 calcium-binding protein B) as well as synaptic proteins such as β -synuclein. In this comment, we aimed to discuss the current scenario of biomarkers in stroke research and their potential uses and limitations for improving patients' care and for better planning clinical trials.

APPLICATIONS OF BIOMARKERS FOR STROKE RESEARCH AND CLINICAL PRACTICE

Current literature evidence suggests that blood-based biomarkers reflecting the severity of brain injury may

aid stroke specialists from primary prevention in the general community or target populations to patients' management during hyperacute and postacute phases as well as for long-term outpatients' monitoring during rehabilitation (Figure). In patients with cardiovascular and metabolic risk factors, such as diabetes⁴ and atrial fibrillation,⁵ elevated serum NfL concentrations were associated with increased stroke risk as a possible indication of silent brain infarctions, which may in turn evolve in a clinically manifest cerebrovascular event.⁶ Similarly, high plasma total tau level may predict the risk of stroke in the general population,⁷ but other chronic conditions at risk such as carotid artery disease are still unaddressed.

When a stroke is suspected, the discrimination between ischemic (acute ischemic stroke [AIS]) and hemorrhagic event (intracerebral hemorrhage or subarachnoidal hemorrhage) is of vital importance for prehospital management, choice of reperfusion strategies, and bleeding control. Rapid access to neuroimaging comes first, but neurochemical markers may help to estimate the risk of intracerebral hemorrhage/subarachnoidal hemorrhage at first medical contact for

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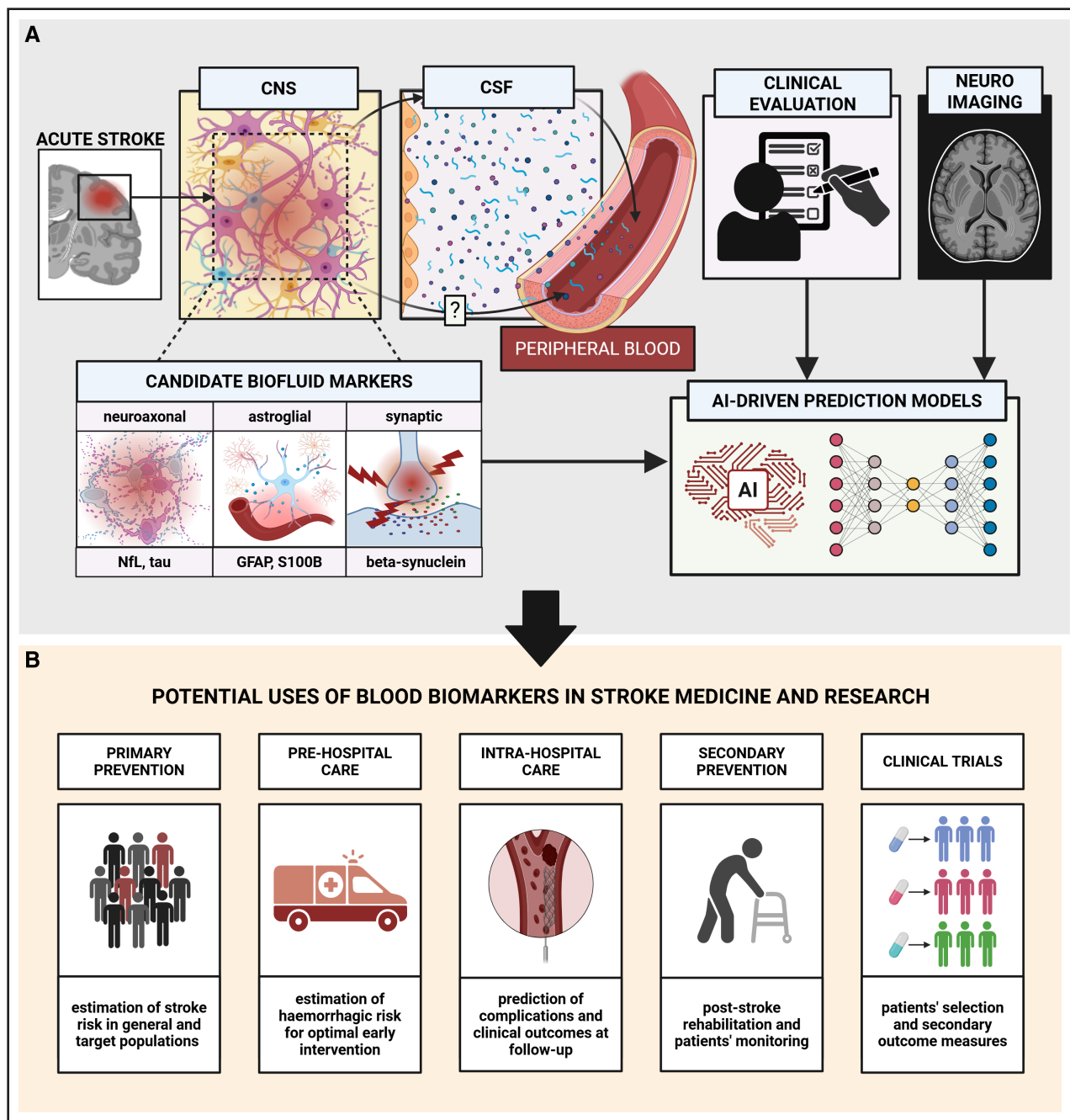


Figure. Potential uses of blood biomarkers of brain injury in stroke clinical care and research.

A, Acute brain injury after a stroke includes phenomena such as neuroaxonal damage, glial activation, and synaptic dysfunction. These events can be detected in peripheral blood by measuring brain-derived proteins which can enter the bloodstream through the cerebrospinal fluid or, hypothetically, also directly. Blood-based biomarkers may be used in combination with clinical evaluation and neuroimaging for developing artificial intelligence (AI)-driven prediction tools. **B**, Possible applications of blood biomarkers of brain injury in stroke clinical practice and research, from primary prevention in the general population to acute stroke care and patients' monitoring in rehabilitation settings to the planning of clinical trials. CNS indicates central nervous system; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NfL, neurofilament light chain; and S100B, S100 calcium-binding protein B.

setting more tailored blood pressure values.⁸ For this purpose, point-of-care platforms allowing biomarker quantification within minutes (eg, GFAP)⁹ seem promising for assessing the prehospital hemorrhagic risk.

More studies focused on the prognostic value of neuronal and glial biomarkers for short- and middle-term

outcomes after stroke as complementary tools alongside clinical and radiological evaluation. Blood NfL and GFAP levels are overall higher in patients with AIS compared with healthy controls and patients with transient ischemic attack.^{2,3,10} High blood level of NfL may indicate a higher risk of all-cause mortality and neurological disability,¹¹⁻¹⁴

poorer cognitive outcomes,¹⁵ poststroke epilepsy,¹⁶ and new magnetic resonance imaging-detected lesions in small vessel disease at follow-up.¹¹ Here, repeated biomarker measurement may track stroke progression, inform on the risk of complications, for example, hemorrhagic transformation after AIS, as demonstrated for both NfL and GFAP,^{17–19} and indicate patients to intensive care regimens. This turns particularly important in critical situations, where decision-makers may prefer to withhold ineffective interventions in case of an expected low probability of survival.²⁰ High serum NfL levels may serve as a proxy of more severe white matter damage/degeneration also for estimating long-term outcomes and stroke recurrence in AIS during rehabilitation phases.²¹ The predictive value of other markers as alternatives or add-on parameters to NfL, either individually or integrated with combined neurochemical profiles,²² still requires systematic assessment.

Because of the complex, multivariable nature of outcome prognostication, biomarkers are probably best integrated into routine care when used to improve validated

prognostic models²³ or to assess novel artificial intelligence-driven prediction models (Figure). Ideally, a panel of biomarkers reflecting distinct faces of brain injury may aid decision-making for identifying patients who may benefit from more aggressive treatment of cardiovascular risk factors as well as specific preventive/rehabilitation programs. These biomarkers can also be adopted as biochemical outcome measures for patients' monitoring in clinical trials (Table). Positive results within trial contexts would further encourage validation studies in real-world settings to evaluate their implementation into routine practice.

MISSING POINTS

Despite its potential, the clinical applicability of blood biomarkers is hampered by several issues still to be addressed. First, the temporal patterns of biomarker concentrations, and consequently the optimal time-point of quantification for each biomarker for diagnostic and prognostic purposes have not been thoroughly

Table. Ongoing Trials on Adult Patients With Stroke Using Blood Biomarkers of Brain Injury as Outcome Measures

Trial identification code	Study location	Design	Study status	Target population	Target intervention	Primary end points	Markers included as outcome measures
NCT05273216	Austria	Observational	Recruiting (study completion estimated in 04/2025)	AIS because of LVO of the anterior cerebral circulation undergoing MT	Transcranial duplex sonography, MRI, blood biomarkers	Rate of intracranial hemorrhage and vasogenic brain edema after MT	NfL and others
NCT03919370	Sweden	Observational	Completed in 12/2024 with unpublished results	AIS undergoing carotid surgery or MT	No intervention	Heart rate variability, near infrared spectroscopy, EEG, biomarkers of brain ischemia, and biomarkers of cardiac ischemia	tau, NfL, GFAP, S100B, NSE and troponin T
NCT04795362	France	Observational	Completed in 06/2024 with unpublished results	SAH	Blood sampling	S100B concentrations after delayed cerebral ischemia suspicion	S100B
NCT06135103	Israel	Interventional	Recruiting (study completion estimated in 09/2025)	AIS due to MCA occlusion recruited after 6–24 mo	Administration of talfirastide (TXA127) or placebo	Motor and sensory function change, adverse events	BDNF, NfL
NCT05836740	China	Interventional	Completed in 08/2024 with unpublished results	Adult patients with AIS	Administration of minocycline or placebo	mRS at 90 d	NfL, S100B and others
NCT05836766	China	Interventional	Recruiting (study completion estimated in 08/2024)	AIS because of LVO of the anterior cerebral circulation	Administration of sublingual Y-6 (cilostazol+dexborneol) or placebo	mRS at 90 d	S100B and others
NCT06116942	Germany	Interventional	Recruiting (study completion estimated in 09/2027)	AIS recruited after 6 mo	Repetitive transcranial magnetic stimulation	MEP parameters, motor and sensory function change, EEG changes	S100B, NfL, GFAP, β -synuclein

Search was conducted on ClinicalTrials.gov by searching as keywords (by April 1, 2025): "stroke" AND "neurofilament light chain" OR "glial fibrillary acidic protein" OR "S100B" OR "NSE" OR "synuclein." AIS indicates acute ischemic stroke; BDNF, brain-derived growth factor; EEG, electroencephalography; GFAP, glial fibrillary acidic protein; LVO, large vessel occlusion; MCA, middle cerebral artery; MEP, motor-evoked potential; MRI, magnetic resonance imaging; mRS, modified Rankin Scale; MT, mechanical thrombectomy; NfL, neurofilament light chain; NSE, neuron-specific enolase; S100B, S100 calcium-binding protein B; SAH, subarachnoid hemorrhage; and TXA127, talfirastide.

assessed. Longitudinal studies on blood NfL found a sustained increase of biomarker levels up to weeks or months after AIS, with concentrations measured at later timepoints showing a higher prognostic accuracy for functional outcome than those obtained soon after clinical onset.^{11,12,24} Late NfL quantification could inform on other aspects of stroke, such as neuroplasticity or secondary neurodegeneration, but little evidence is present to date. The longitudinal changes of other markers in comparison with NfL, such as GFAP, have only been preliminarily explored.²⁵ Second, the interpretation of brain-derived biomarkers may be misled by comorbidities influencing biomarker levels independently from stroke but which are highly prevalent in the stroke population, for example, impaired renal function, uncontrolled high blood pressure, or HbA1c values but also genetic and social elements.^{2–4,20} The definition of pathological biomarker thresholds and the dichotomization into normal/abnormal categories could ease adoption in routine practice, but this approach lacks analytic efficacy and pathophysiological plausibility.^{23,26} Furthermore, the standardized quantification methods broadly accessible need to be evaluated in cost-benefit analyses on a large scale. These issues may be overcome with correction formulas, normalized values with reference populations,²⁷ or the validation of novel biomarkers. As an emerging biomarker, β -synuclein was the first blood marker described to monitor synaptic damage after AIS, and it showed preliminarily a high prognostic value and low associations with systemic confounding factors.^{14,28} In comparison with other markers such as NfL and GFAP, synaptic proteins could present theoretical advantages such as rapid dynamics over time and a closer relationship with the extent of cortical damage, thus being more informative on the neurological outcome. Comparison studies with biomarkers thoroughly examined in the past years, for example, S100B,²⁹ are required to evaluate how clinically meaningful these novel biomarkers are for stroke specialists. Third, blood amyloid- β peptides and phosphorylated tau proteins, which are first-line markers for assessing Alzheimer disease pathology, have been explored in patients with stroke, with more elevated biomarker concentrations being associated with poorer functional outcomes at follow-up.³⁰ However, the predictive value of such markers for cognitive impairment and dementia after stroke remains undressed. Fourth, preliminary findings in AIS and intracerebral hemorrhage need validation in other conditions associated with stroke, such as cerebral amyloid angiopathy,³¹ as well as in nonwestern populations, which are currently underrepresented in biomarker³² and stroke research.³³ Here, region-specific investigations, multicentric studies, and assessments of ethnicity-related factors underlying biomarker level diversity may help fill this gap.

CONCLUSIONS AND FUTURE PERSPECTIVES

The implementation of blood markers in stroke medicine has great potential for improving patients' care from primary prevention to poststroke monitoring and trial design. Research findings encourage the additional use of biofluid markers of brain injury in addition to standard clinical end radiological evaluation for rapidly estimating the risk of complications and the clinical outcome. Methodological limitations, estimated costs, and best biomarker combinations need to be evaluated in real-world studies to ponder net clinical benefit.

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