Blood Biomarkers in the Diagnosis of Acute Stroke

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8.1 Introduction

Acute stroke represents a leading cause of death in industrialized countries and has a considerable socioeconomic impact due to its attached high morbidity leaving up to 50% of the survivors chronically disabled [1].

Two pathophysiological types of stroke can be distinguished: the ischemic stroke due to an occlusion of an arterial blood vessel leading to local oxygen undersupply of the brain tissue and the hemorrhagic stroke, which is caused by a disruption of the integrity of a vascular wall within the brain with subsequent extravasation of blood into the surrounding tissue or subarachnoid space, potentially leading to neuronal demise [2].

Despite the intuitiveness of these underlying pathophysiological mechanisms, the diagnostic and therapeutic workup in cases of suspected stroke holds several difficulties: Acute stroke represents a heterogeneous syndrome with a variety of clinical manifestations encompassing evident deficits, such as acute facial or limb weakness, but also more subtle, unspecific symptoms such as dizziness, gait disturbance, or severe headache. As the absence of the mentioned symptoms above does not safely preclude a severe cerebrovascular event, every patient with newly developed focal neurological deficits requires prompt radiological evaluation [3, 4].

The distinction between ischemic and hemorrhagic stroke is crucial as their therapeutic approaches are diametrical: systemic thrombolysis with recombinant tissue plasminogen activator (rt-PA) represents a therapeutic core element in cases of acute occlusions of intracranial arteries due to its ability to restore cerebral blood supply by the dissolution of a blood clot. In contrast, hemodilution in cases of

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intracranial hemorrhage may fuel its growth and is therefore strictly contraindicated. In this context, time is the most critical factor especially in cases of large vessel occlusion, as the potentially salvageable brain tissue gradually diminishes over time leading to a narrow therapeutic window with an increased risk for neurologic sequelae and worse outcome in cases of treatment delay [5, 6].

Current diagnostic algorithms recommend computed tomography (CT) or magnetic resonance imaging (MRI) of the head to rule out intracranial hemorrhage and to determine whether there is an underlying vascular occlusion.

Nevertheless, unremarkable initial brain imaging findings do not rule out a transient insufficient blood supply or lacunar ischemic event as the underlying cause for observed neurologic deficits. Against this background, the differentiation between a causative vascular problem and a stroke mimic (such as migraine, hypertensive crisis, or epileptic seizures) can be difficult [7].

In recent years, interest in the field of novel biomarker research has steadily increased. Biomarkers are generally referred to as objective indicators of an individual's (patho-) biological state, which can be measured reproducibly and accurately [8]. Studied areas of application within stroke biomarker research involve the rapid biomarker-based differentiation between stroke subtypes and stroke mimics to further streamline the prehospital management in patients with suspected stroke and prevent imminent treatment delay in cases where clinical assessment is difficult and immediate neuroimaging is not readily available [7, 9]. In the broadest sense, these stroke biomarkers can be considered as candidate surrogate markers in the diagnostic context [10].

This chapter provides an overview of the biomarkers that are currently the focus of research, enters into the details of clinically promising candidates, and discusses potential areas of their application from a clinical point of view.

8.2 Overview of Emerging Biomarkers for Risk Stratification, Diagnosis, and Etiological Classification in Acute Stroke

There is a wide range of promising candidates that are currently subject to research. Table 8.1 presents an overview of biomarkers with reported potential diagnostic use in a broader or narrower sense without claiming to be exhaustive.

Table 8.1 Overview of biomarkers for risk stratification, diagnosis, and etiological classification in acute stroke (adapted from [7, 9, 11–21])

Biomarker group	Biomarker	Description
Associated with glial cells	S100β (calcium-binding protein-beta)	Calcium-binding protein expressed by astrocytes and oligodendrocytes
	GFAP (glial fibrillary acidic protein)	Intermediate filament protein predominantly expressed by astrocytes

Table 8.1 (continued)

Biomarker group	Biomarker	Description
Associated with neuronal cells	Serum neurofilament light chain (SNfL)	Polypeptide filaments, component of the axonal cytoskeleton
	NSE (neuron-specific enolase)	Dimeric glycolytic isoenzyme in the cytoplasm of neurons/neuroendocrine cells
	HFABP (heart fatty acid-binding protein)	Cytosolic protein that modulates lipid signaling cascades; involved in intracellular fatty acid transport
	MBP (myelin basic protein)	Main proteolipid constituent of myelin, produced by oligodendroglia cells
	Tau	Protein that stabilizes microtubules and assists with axonal maintenance and transport in neurons
	VLP-1 (visinin-like protein-1)	Member of the family of neuronal intracellular calcium sensor visinin-like proteins, part of the calcium-dependent cell signaling involved in the modulation of cAMP (cyclic adenosine monophosphate)
	NMDA-receptor antibodies (NR2A/NR2B subunits of the NMDA receptor)	Excitotoxic receptor
Associated with hemostasis	D-dimer	Fibrin degradation product that reflects global activation of coagulation and fibrinolysis
	vWf (von Willebrand factor)	Adhesive glycoprotein involved in factor VIII platelet adhesion stabilization
	Fibrinogen	Acute-phase protein, involved in leukocyte-endothelial interaction, platelet aggregation, and hemostasis
Associated with inflammation	CRP (C-reactive protein)	Acute-phase protein, part of innate immune response
	Cytokines (TNF-a, interleukin-1b/-6)	Inflammatory cytokines
	Matrix metalloproteinases (MMP-2, MMP-9)	MMP-2/-9: Proteolytic enzymes from the family of gelatinases; possess the ability to activate pro-inflammatory cytokines
	Lipoprotein-associated phospholipase A2 (Lp-PLA2)	Hydrolytic enzyme
	Adhesion molecules (VCAM-1 [vascular cellular adhesion molecule], ICAM-1)	Immunoglobulin superfamily members VCAM-1: Binds monocytes and lymphocytes
	ApoC-1 (apolipoprotein C-1), ApoC-3 (apolipoprotein C-3)	ApoC-1: Associated with LDL and VLDL, involved in plasma lipoprotein remodeling, inhibits CETP ApoC-3: Associated with VLDL, HDL, and LDL; inhibits triglyceride hydrolysis by lipoprotein/hepatic lipase; interferes with normal endothelial function

(continued)

Table 8.1 (continued)

Biomarker group	Biomarker	Description
Associated with cardiac function	Natriuretic peptides (ANP [atrial natriuretic peptide], BNP [brain natriuretic peptide]/NT-proBNP [N-terminal-pro B-type natriuretic peptide])	Myocardial polypeptides with natriuretic, diuretic, and vasodilator activity
Associated with oxidative stress	PARK7	Redox-sensitive molecular chaperone
Other biomarkers	NDKA (nucleoside diphosphate kinase A)	Protein kinase
	Brain-derived neurotrophic growth factor	Neurotrophin superfamily member; growth factor proteins important for neuronal development and function
	Fibrillin-1	Glycoprotein and important component of elastic fibers

8.3 Blood Biomarkers in the Diagnosis of Acute Stroke: A Clinical Perspective

8.3.1 Background

Through a series of large-scale genome, proteome, and metabolome sequencing, a multitude of promising molecules linked to different pathophysiological steps of the stroke cascade were identified (see Table 8.1) [7]. Studies have shown that a small proportion of proteins, albeit to a varying extent, are capable to distinguish between patients with and without stroke [7]. Nevertheless, there are no blood biomarkers for acute stroke used in daily clinical routine for diagnostic purposes to the present day [7, 22].

The reason is that a diagnostic biomarker has to meet different requirements to be used in clinical diagnostics. Ideally, markers should be easily measurable in the course of the first routine point-of-care blood testing, such as blood glucose or the international normalized ratio (INR) [7, 23]. Stroke markers should be quickly released from the brain tissue after neuronal damage occurred, pass the blood-brain barrier rapidly, and be dispensed steadily into the peripheral blood to be detectable soon after stroke onset [16]. A crucial demand for a diagnostic stroke biomarker is its ability to reliably separate affected from healthy individuals. Key indicators in this respect are sensitivity and specificity. A sensitivity of 90% for the detection of an acute ischemic stroke would still mean that 10% of patients with ischemic stroke would mistakenly remain undetected, potentially impeding a rapid initiation of treatment. On the contrary, a specificity of 90% would mean that 10% of the tested healthy individuals would falsely be identified as sick, entailing the risk of over-treatment [7]. Besides the desired high sensitivity to early cerebral damage and the

specificity of a stroke biomarker candidate for the brain tissue, the key question in clinical context due to its attached therapeutic implications is: Can the biomarker candidate distinguish between ischemic and hemorrhagic stroke with sufficient reliability [7, 17]? In hemorrhagic stroke, the ability to make this distinction could allow early antagonization of oral anticoagulants and early initiation of antihypertensive therapy [24, 25]. In acute ischemic stroke, a reliable preclinical biomarker-based identification could facilitate the rapid assignment to a stroke center and therefore potentially increase the proportion of patients treated with intravenous thrombolysis and endovascular treatment, respectively, ensuring the most beneficial outcome [9, 18, 24]. Furthermore, in cases of unclear time of symptom onset, which accounts for up to 28% of all patients with acute ischemic stroke, the release kinetics of a diagnostic biomarker or its detection with known delayed release could help to deduce the approximate beginning of the event and help guide therapeutic decision-making [9, 26].

8.3.2 Biomarkers for the Early Differentiation Between Acute Cerebrovascular Events and Mimicking Conditions

8.3.2.1 *N*-Methyl-D-Aspartate (NMDA) Receptor

NMDA receptors bind the neurotransmitter glutamate. Elevated levels of glutamate mediate excitatory effects through the activation of NMDA receptors within the pathophysiologic ischemic stroke cascade, ultimately leading to neuronal damage [27–29]. The NMDA receptor in its basic configuration consists of 4–5 units (2 NR1 and 2–3 NR2), with NR2A and NR2B as the two major subunits of NR2 in adult neocortex [30–32].

In the course of cerebral ischemia, an autoimmune-mediated formation of antibodies against NMDA receptor peptide fractions has been demonstrated in the peripheral blood [29, 33]. NR2A/2B autoantibody concentrations were significantly higher in patients with transient ischemic attack (TIA) and acute ischemic stroke compared to controls [30]. The sensitivities for the diagnosis of ischemic strokes and TIAs within 3 h after symptom onset using a cutoff of 2.0 μg/L were 97% and 95%, respectively. The specificity for both entities was 98% [30].

Differences within the temporal course of NR2A antibody elevations have been demonstrated in cases of ischemic stroke with a concentration peak around 9 h after hospital admission [28]. Interestingly, the antibody concentrations were higher in infarcts involving the brain cortex [28]. As demonstrated by another study, the formation of NMDA receptor antibodies is also detectable in cases of intracranial hemorrhage suggesting that a reliable differentiation from patients with ischemic stroke is not possible through the sole evidence of antibodies in the serum [30]. Nevertheless, regarding the time course of the concentration changes in the serum, peak values were achieved earlier and were lower in patients with intracranial hemorrhage compared to patients with acute ischemic stroke [30]. The latency between symptom onset and the achievement of peak concentrations ranged between 9 and 12 h for patients with acute ischemic stroke and 3 and 5 h for patients with intracranial

hemorrhage [30]. The positive predictive value for ischemic strokes was 86% and 91% for TIAs, and the negative predictive value was 98% for both entities [30].

The measurement of NMDA receptor antibodies might be helpful to discriminate between patients with TIAs and stroke mimics [30]. However, the additional informative value of NMDA receptor antibodies in the emergency setting remains uncertain. A study with 120 patients presenting with acute ischemic stroke or TIA within 72 h after symptom onset showed that men and women with a history of stroke in the prior 6 months had elevated NR2 antibody levels compared to controls, potentially reducing its informative significance for the detection of recent ischemic cerebral events in patients with a history of stroke or TIA, respectively [34].

Another issue in the diagnostic context is the expected relatively long latency period between the formation of antibodies and their detectability in the peripheral blood, leading to a shift of interest towards the NR2 peptides, which are thought to be produced earlier after the ischemic event [16].

8.3.2.2 Lipoprotein-Associated Phospholipase A2 (Lp-PLA2)

Lp-PLA2 is an enzyme that catalyzes the conversion of low-density lipoprotein into proinflammatory metabolites and is found to be predominantly expressed in macrophage-containing atherosclerotic lesions [35, 36]. Lp-PLA2 is considered a marker for vascular inflammation [35, 37]. The pathophysiological linkage between Lp-PLA2 and occurrence of ischemic stroke is likely to be that inflammation, as a recognized underlying mechanism for the development of atherosclerosis including the rupture of unstable plaques, may ultimately lead to cerebrovascular events [38–40]. Lp-PLA2 mainly circulates bound to LDL in the peripheral blood with a small fraction binding to HDL [39]. A proof-of-concept trial with individuals with previous ischemic stroke under therapy with statins demonstrated lower Lp-PLA2 mass levels compared to individuals with ischemic stroke without established statin therapy [41]. Nevertheless, Lp-PLA2 and LDL cholesterol levels appear to be independent of each other [39, 42].

Lp-PLA2 activity was demonstrated to predict ischemic stroke and coronary heart disease in the general population [35, 43]. Lp-PLA2 mass and activity showed to be associated with symptomatic large vessel stenosis, but not with cardioembolism [40]. A recent clinical trial investigated the relation between intima-media thickness of the carotids and Lp-PLA2 blood levels in cases of arterioembolic stroke [44]. Although not reaching the threshold of statistical significance, Lp-PLA2 blood levels were correlated with the intima-media thickness in patients with arteriosclerotic ischemic stroke supporting the previously suspected pathophysiological linkage [44].

Additionally, Lp-PLA2 mass levels were shown to predict recurrent stroke in individuals with known stroke history [35]. Although the predictive value of Lp-PLA2 activity for recurrent stroke seems to be limited to individuals with LDL levels below 130 mg/dL, suggesting a possible interaction of the Lp-PLA2 activity and LDL levels, Lp-PLA2 is widely considered as an independent predictor for stroke [35, 38, 39].

Furthermore, Lp-PLA2 proved to add valuable information to the risk stratification in patients with TIAs [40]. Patients with high Lp-PLA2 activity and mass levels exposed a higher risk for subsequent stroke and death, classified as members of the moderate-risk group using the $ABCD_2$ score before [40].

There is still a certain heterogeneity regarding the measurement of Lp-PLA2. This is likely due to different assays that measure either the mass or the activity of Lp-PLA2, causing a variability since Lp-PLA2 mass and activity do not strictly correlate [45]. The main reason for this limited correlation is thought to be that mass tests mainly detect the HDL-associated Lp-PLA2, which represents only a small proportion of the total Lp-PLA2 [45]. Altogether, it remains unclear to which extent the observed incomplete correlation between measurable Lp-PLA2 activity and mass is attributable to the enzymes' biochemical characteristics or technical aspects of the available assays [35]. Further studies investigating the temporal release kinetics of Lp-PLA2 are needed.

8.3.2.3 Heart-Type Fatty Acid-Binding Protein (HFABP)

HFABP belongs to a family of proteins with different tissue distribution patterns [46]. It is found in the brain, but also the heart, the lung, and the kidneys [46]. This family of proteins plays an important role in maintaining cellular homeostasis [46].

A pilot study investigated the ability of HFABP to discriminate between patients with stroke, patients with myocardial infarction, and healthy controls [46]. The specificity and sensitivity of HFABP for the diagnosis of acute stroke were 100% and approximately 68%, respectively [46]. However, no differentiation between hemorrhagic and ischemic stroke was possible [46].

Moreover, there is evidence that HFABP serum levels within the 6 h after stroke onset correlate with the severity of symptoms in patients with acute ischemic stroke [47]. As expected, elevated HFABP serum levels were also observed in patients with acute myocardial infarction, which might pose a diagnostic obstacle in acute clinical situations [46]. The relatively low sensitivity would call for a multi-marker approach in a clinical setting [47].

A potential benefit of HFABP is its relatively fast release into the blood with measurable elevations within a few hours after stroke onset [46, 47]. Overall, the evidence regarding the potential clinical benefit of HFABP is still too limited—only a few studies with a low number of cases exist—to be able to make general statements about possible fields of application in clinical routine.

8.3.2.4 Parkinson Disease Protein 7 (PARK 7)

The gene coding for PARK 7 was discovered as an autosomal recessive gene related to a form of familial Parkinson's disease [48–50]. Although expressed in the cerebral tissue where it is assumed to have a reparative function in cellular damage caused by oxidative stress, PARK 7 does not appear to be specific for the central nervous system [48, 50]. The exact function of PARK 7 and its release mechanisms are not entirely understood [48, 50]. Yet it is plausible to assume that neuronal damage in the context of acute stroke leads to a release of the protein to the systemic circulation through direct penetration of the disrupted blood-brain barrier [48].

This assumption is supported by a large-scale three-center study demonstrating a significant release of PARK 7 into the systemic circulation within 30 min to 3 h after symptom onset in patients with acute ischemic (including TIA) and hemorrhagic stroke [48].

The diagnostic sensitivity for the diagnosis of acute stroke (i.e., hemorrhagic, ischemic, and TIA) varied between 54% and 91%, and the specificity between 80% and 97%, depending on the used cutoffs and the timeframe between symptom onset and time of blood sample collection (range of 30 min to 5 days) [48]. As the previous data implicates, discrimination between stroke subtypes and conditions that may mimic stroke using (serial) PARK 7 measurements is currently not possible [48].

8.3.3 Biomarkers for the Differentiation Between Ischemic and Hemorrhagic Stroke

8.3.3.1 Glial Fibrillary Acidic Protein (GFAP)

One of the most promising markers for stroke diagnosis is GFAP. It is a monomeric filament protein that is relatively specific for astrocytic glial brain cells [51, 52]. Various studies suggest its potential use for the differentiation of stroke subtypes as GFAP serum levels were shown to be elevated in patients with ischemic stroke and intracranial hemorrhage compared to healthy subjects and other neurological conditions [51, 53].

One of the first larger studies including 135 patients with first-time acute stroke, ischemic or hemorrhagic, evaluated the ability of GFAP to differentiate between both stroke entities within 6 h after symptom onset [54]. The analysis of the serum GFAP levels at admission revealed that 81% of patients with intracranial hemorrhage demonstrated measurable GFAP-level elevations, while only 5% of patients with ischemic stroke showed an increase over the detection threshold [54]. The diagnostic specificity of GFAP for the identification of intracranial hemorrhage in this mixed stroke cohort within the first 6 h after symptom onset was 98%, and the sensitivity 79% at a cutoff point of 2.9 ng/L [54]. The overall diagnostic accuracy of GFAP for the differentiation between intracranial hemorrhage and acute ischemic stroke within a timeframe of 3 h after symptom onset was 91% [54].

A subsequent multicenter study with 202 patients with radiologically confirmed acute ischemic stroke or intracranial hemorrhage, who were admitted to the hospital in less than 4.5 h after symptom onset, evaluated the ability of GFAP to differentiate between both stroke entities [24]. Patients with prior ischemic or hemorrhagic stroke or other preexisting neurological disorders were excluded [24]. The diagnostic specificity of GFAP for the differentiation between ischemic stroke and intracranial hemorrhage in this cohort within the first 4.5 h after symptom onset was 96.3%, and sensitivity 84.2% [24]. The GFAP levels in patients with intracranial hemorrhage correlated with the NIHSS (National Institutes of Health Stroke Scale) scores at admission, most likely reflecting the tissue damage-dependent GFAP release into the blood [24, 55].

However, the specifications vary across different studies considering the different times of specimen collections and cutoffs used [25, 54, 56, 57]. A research project further examining the release kinetics of GFAP demonstrated an accuracy of over 80% in the timeframe of 2–6 h after symptom onset, whereby GFAP levels correlated significantly with the hemorrhage volume in cases of intracranial bleeding 2 h after symptom onset [56].

On the contrary, the prolonged release of GFAP into the blood reaches the maximum concentration between 48 and 96 h after event onset in cases of ischemic stroke [54]. Therefore, no correlation between the initial GFAP levels and symptom severity can be seen in cases of ischemic stroke [24, 55]. In this context, it becomes evident why transient hypoperfusion not leading to permanent structural damage does not necessarily translate into elevated GFAP levels in the peripheral blood [24, 55, 58]. It should also be taken into account that GFAP baseline blood levels may vary by age, as there is evidence derived of cerebrospinal fluid samples of 25 subjects with no history or current evidence for neurological disorders that shows an increase with age [59].

As indicated above, the release kinetics are thought to be different depending on the stroke subtype with a suspected faster release of GFAP due to immediate astrocytic damage and a consecutive timely disruption of the blood-brain barrier in cases of intracranial hemorrhage [24, 25, 54]. Consequently, the available upper time limit in which high GFAP levels are likely attributable to intracranial hemorrhage is 6–12 h after the onset of stroke symptoms [24, 25].

A Swedish multicenter study measured several biomarker levels in a mixed stroke cohort of 97 patients within 24 h of symptom onset. Interestingly, the combination of GFAP with another blood biomarker called APC-PCI (activated protein C–protein C inhibitor complex) revealed a negative predictive value of 100% for intracranial hemorrhage in patients with NIHSS scores of more than 3, compared to the previous stated negative predictive value of 91% with GFAP alone [53, 54].

Yet, there is still a fundamental problem concerning the use of GFAP in clinical routine. The knowledge of how concomitant cerebral pathologies potentially leading to cerebral gliosis themselves might influence the release of GFAP into the bloodstream is limited [24]. Moreover, the observation that GFAP was not detectable in the wide majority of patients with acute ischemic stroke within the time window of 6 h after symptom onset reduces its informative value in acute management [54]. It is also noteworthy that data about GFAP concentrations and their temporal release in cases of lacunar ischemic stroke or cerebral microbleeds, especially infratentorial, is still scarce [24]. One study investigated several biomarkers concerning their ability to differentiate vertigo due to posterior circulation stroke from the vertigo of nonvascular origin [60]. The vast majority of patients suffering from vertigo due to an infratentorial hemorrhage did not show elevated GFAP blood levels within the first 24 h after symptom onset [60]. A convincing explanatory approach is the already previously described correlation between GFAP release and lesion size [60].

Even though increases of GFAP concentrations emerge earlier compared to other astroglial markers such as S100β in cases of acute ischemia in the anterior

circulation, an important limitation for the use of GFAP as a point-of-care test remains the limited discriminatory power between intracranial hemorrhage and lacunar stroke in cases of unknown time of symptom onset [56, 61]. Given these contradictory findings regarding the sensitivity of GFAP for the early detection of intracranial hemorrhage within a 0–2 h after symptom onset, further studies are needed to clarify the diagnostic accuracy in this timeframe [24, 56].

8.3.3.2 S100 Calcium-Binding Protein β (S100β)

S100β is part of the cytosol of astrocytic glial brain cells, consists of two subunits (α and β), and is specific to the nervous system [52, 62–64]. The history of the name goes back to the 1960s and is derived from its solubility in a saturated ammonium sulfate solution [65]. Interestingly, S100β-expressing cells do not express GFAP, although both being glial typical markers, due to different developmental stages of the brain astrocytes [66]. Mainly intracellularly localized, S100β has a variety of regulatory functions in the calcium homeostasis, cell proliferation, and apoptosis [64, 67].

S100β levels were significantly higher in stroke cohorts compared to healthy controls [64, 68, 69]. However, elevated S100β serum levels have not exclusively been reported in patients with TIAs and ischemic and hemorrhagic stroke, but also in context with other neurological conditions such as traumatic brain injury, migraine, and neurodegenerative diseases [69–73]. Surprisingly, significant differences regarding the temporal course were observed between patients with ischemic stroke, TIA, and traumatic brain injury suggesting different underlying release mechanisms [72].

Whether S100β can distinguish between ischemic and hemorrhagic stroke has been a matter of ongoing debate. A large Spanish study addressed this question using a biomarker-based approach with a total number of 915 patients with confirmed acute ischemic or hemorrhagic stroke [74]. The blood biomarker panel contained, among others, CRP, D-dimer, S100β, sRAGE (soluble receptor for advanced glycation end products), MMP-9, and BNP [74]. Blood samples were collected within 6 h after event onset [74]. As a result, within the tested panel, increased S100β and decreased sRAGE levels were associated with intracranial hemorrhage, emphasizing its potential distinctive ability towards patients with ischemic stroke [74]. Even though the exact release mechanisms are still not understood, the authors assume that an early release in cases of intracranial bleeding as observed with GFAP, another glial marker, is due to faster cell destruction compared to the gradual neuronal demise associated with ischemic damage [74].

Despite these promising results, S100β failed to discriminate between ischemic stroke and intracranial hemorrhage within the first 24 h after symptom onset in an explorative biomarker analysis containing 97 patients with acute stroke [53].

On the other end of the spectrum, a more recent study with a total of 142 stroke patients, including TIAs, came to a different result [69]. Higher S100β serum levels were reported in patients admitted within 48 h after symptom onset in cases of cerebral ischemia compared to patients with intracranial hemorrhage and TIAs [69]. Remarkably, the proportion of individuals with intracranial hemorrhage was

relatively high with almost 25%, exceeding the proportion of the previous studies by approximately 10% [69].

To elucidate these seemingly contradictory findings which may partly be due to different times of sample collections, heterogeneous cohorts, or technical measurement aspects, understanding the release kinetics of S100β is particularly important.

The half-life of S100β is approximately around 30 min, indicating that a measurable elevation over days is associated with an ongoing neuronal injury [70]. Overall, the release kinetics of S100β seem to be strongly influenced by the duration of the astrocytic functional disruption as well as the site and the extent of neuronal damage [75]. The first studies to determine the time course of S100β levels in the peripheral blood in patients with acute ischemic stroke stated a maximum increase of S100β serum levels 2–3 days after symptom onset [68, 76, 77]. Notably, ischemic stroke due to mainstem or multiple branch occlusion lasting for more than 6 h showed significantly higher S100β levels than patients with a single branch or smaller artery occlusions [75]. In addition, there is some evidence that cortical infarctions lead to earlier elevations of serum S100β levels compared to subcortical or brainstem ischemic events [64]. In patients with ischemic stroke, the S100β peak level reflected the NIHSS score at admission as well as the changes in NIHSS score over the first 10 days after symptom onset [72]. Increments of S100β plasma levels were shown to be associated with larger infarct volume in cases of ischemic stroke and unfavorable outcomes in cases of ischemic and hemorrhagic stroke [63, 64, 75]. According to a study with a cohort of patients with proximal occlusions of the middle cerebral artery, S100β predicted a malignant course defined as the evidence of space-occupying edema with possible subsequent herniation [62]. A large-scale study investigating the initial blood samples of patients that were admitted with acute ischemic stroke and received treatment with rt-PA demonstrated S100β elevations within the first 24 h after symptom onset [78]. Interestingly, the serial analysis of blood samples 2 and 24 h after the administration of rt-PA did not show significant differences regarding the concentrations of S100β [78]. An almost simultaneously published study confirmed the early elevation of S100β after stroke onset [79].

A comparison of the temporal profiles of S100β level changes within 3 days after admission showed a significant difference between stroke patients and patients with traumatic brain injuries and TIAs, respectively [72]. Furthermore, no differences of serum S100β levels on admission were reported between cases of confirmed stroke when compared to those with TIAs, brain metastasis, or hypertensive emergencies [80]. Despite the uncertainties, this finding suggests different underlying pathophysiological mechanisms of damage in each entity [72].

In contrast to other biomarkers such as GFAP and MMP-9, S100β showed the potential to discriminate patients with ischemic and hemorrhagic stroke in the posterior circulation from patients without acute vascular events [60]. A recent study investigated whether S100β, alone and in combination with copeptin, was able to preclude a cerebrovascular event in patients presenting at the emergency department for newly occurred dizziness [81]. S100β levels were shown to be significantly

higher in patients with stroke as the suspected underlying cause for the new episode of dizziness [81]. The negative predicate value of S100β alone for stroke was 95% at a diagnostic sensitivity of 54%, and specificity of 97% [81].

8.3.4 Biomarkers for the Prediction of Clinical Severity and Complications in Acute Stroke

8.3.4.1 Matrix Metalloproteinase 9 (MMP-9)

MMP-9 belongs to a group of proteolytic enzymes that are involved in remodeling processes of the extracellular matrix through their ability to cleave collagen and laminin, representing two of the main components of basement membranes [82–84].

The overexpression and capability of MMP-9 to deteriorate components of the basal membrane are widely considered as an explanatory approach for the disruption of the blood-brain barrier entailing perifocal edema, inflammatory response, and ultimately neuronal demise in the context of stroke [82, 83, 85, 86]. Elevated MMP-9 levels were observed in ischemic stroke, treated [82] or not treated with rt-PA [85], as well as in cases of hemorrhagic stroke [83]. High MMP-9 levels seem to be associated with a higher degree of neurological impairment and a larger infarct volume within the first 48 h of acute ischemic stroke [85].

The temporal course of MMP-9 levels was observed in patients with ischemic stroke due to an occlusion of the middle cerebral artery treated with rt-PA within the first 3 h after symptom onset [82]. Consistent with the supposed role of MMP-9 for the preservation of the blood-brain barrier, the pretreatment MMP-9 levels predicted intraparenchymal hemorrhage in acute ischemic stroke after applying thrombolytic therapy in those patients [82].

Apart from the administration of thrombolytic therapy, a study conducted on 250 patients with acute hemispheric ischemic stroke demonstrated an association between elevated MMP-9 levels within the first 24 h after symptom onset and hemorrhagic transformation [87]. The sensitivity and specificity of MMP-9 levels from 140 ng/mL upwards for the prediction of hemorrhagic transformation were around 90%, whereas the negative predictive value was 97% [87]. Unfortunately, it is still not clear whether elevated MMP-9 serum levels during the first 4.5 h after stroke onset are robust predictors of secondary hemorrhage within this critical early phase [87].

Interestingly, a subsequent study of the blood levels of cellular fibronectin (c-Fn), an adhesive protein primarily produced by endothelial cells, in patients with acute ischemic stroke treated with rt-PA showed a significant association with hemorrhagic transformation [86]. Although a positive correlation between c-Fn and MMP-9 blood levels was observed, the sensitivity (100%), specificity (96%), and negative predictive value (100%) of c-Fn for the prediction of hemorrhagic transformation at a cutoff of 3.6 μ/mL were shown to be even higher in comparison to those of MMP-9 [86]. Moreover, the applied logistic regression models only revealed c-Fn levels to be independently associated with hemorrhagic transformation,

indicating its potential superiority compared to MMP-9 regarding its diagnostic value in rt-PA-associated intracranial bleedings [86].

A further study examining the temporal profiles of different metalloproteinases including MMP-9 after spontaneous intracranial bleeding showed dynamic changes within the further course [83]. Baseline MMP-9 blood levels were related to the radiologically determined hemorrhage-associated edema and the residual scar volume at 3 months [83].

8.3.4.2 Myelin Basic Protein (MBP)

MBP is a myelin membrane protein with a structural role within the myelin sheaths of Schwann cells and oligodendrocytes [78, 88, 89]. Analogous to S100β, increasing serum MBP concentrations were reported in cases of ischemic stroke within the first 24 h after symptom onset [78]. A comparison of serum MBP levels between patients with radiologically confirmed acute ischemic stroke, obtained within the first 12 h after symptom onset, and a healthy control group did not show a significant difference [88].

A smaller study investigated the admission levels of different stroke biomarkers, including MBP, in a heterogeneous cohort of 28 patients with acute ischemic stroke [90]. Among these patients, 1 had a total anterior circulation stroke, 10 had partial anterior circulation stroke, 8 had lacunar ischemic stroke, 4 had posterior circulation stroke, and 5 had unknown types [90]. Overall, the analysis showed elevated MBP levels on admission in 39% of patients [90].

Although MBP might not qualify as a sufficiently accurate diagnostic biomarker in early stroke, the release kinetics may provide additional information. Higher MBP peak concentrations within the first days were associated with the higher NIHSS baseline scores and larger lesion volume in the CT brain scans [78]. Moreover, smaller changes in MBP levels within the first 24 h were observed in patients demonstrating favorable outcomes [78].

8.3.5 Biomarkers for the Etiological Classification of Ischemic Cerebrovascular Events

8.3.5.1 Natriuretic Peptides (ANP/BNP)

Atrial (ANP) and brain natriuretic peptides (BNP) are co-released mainly by the cardiac atriums and ventricles in response to an increased myocardial wall tension and hemodynamic stress but remarkably also in the course of acute ischemic stroke, promoting vasodilatation and natriuresis [73, 91–96].

Besides being expressed by cardiomyocytes, ANP is also found within the central nervous systems (i.e., hypothalamus and septum) [97]. In recent years, there has been growing interest in the midregional fragment of the precursor hormone of ANP (midregional proANP; MR-proANP) due to its higher sample stability [98]. In a large multicenter study including over 700 patients with acute ischemic stroke high MR-proANP levels were independently associated with cardioembolic stroke

etiology and atrial fibrillation [97, 99]. Moreover, high levels of MR-proANP are associated with small vessel infarcts and white matter lesions on MRI [100].

The name BNP is derived from the fact that this peptide was first isolated from porcine brain tissue [92]. ProBNP represents the precursor molecule of BNP, whereby inactive N-terminal peptides (NT-proBNP; N-terminal pro-brain natriuretic peptide) are released in the course of the cleavage process [93]. The fact that the half-life of NT-proBNP is longer compared to that of BNP makes it preferable for measuring purposes [93].

NT-proBNP blood levels were found to be elevated in patients with acute ischemic stroke within the first 24 h after symptom onset independently of echocardiographic parameters [91]. The admission levels were significantly higher in cases of cardioembolic origin [91]. A meta-analysis comparing circulating BNP/NT-proBNP levels across different stroke etiologies with data from over 2800 patients demonstrated significant increments of BNP/NT-proBNP in cardioembolic ischemic stroke within the first 72 h after stroke onset compared to patients with non-cardioembolic stroke [93].

These findings are in line with other reports of an observed strong association between elevated NT-proBNP levels and an increased risk of cardioembolic stroke in the general population [101]. BNP secretion is believed to be induced due to its vasodilatory effect as a counter-regulation mechanism in cases of ischemic stroke [91]. Independent from the occurrence of an ischemic stroke, there is evidence that increased NT-proBNP levels are associated with the presence of atrial fibrillation [93, 101, 102]. In patients with chronic non-valvular atrial fibrillation, BNP represents an independent predictor of thromboembolic complications as BNP blood levels were demonstrated to be higher in patients with thromboembolic events compared to individuals without complications [95]. Following this, high proBNP levels predicted the occurrence of atrial fibrillation within 2 years after cryptogenic stroke [103]. Although the mechanisms are not clear, BNP elevations in the peripheral blood have also been reported in patients with subarachnoid hemorrhage [91].

Overall, data comparing the performance of NT-proBNP and MR-proANP for the prediction of cardioembolic etiology in the context of ischemic stroke are limited.

8.3.5.2 D-Dimer

D-dimer represents a product of plasmin-mediated fibrin degradation and typically indicates thrombus formation [30, 104–106]. Elevated D-dimer levels were found ubiquitously in cardioembolic and atherothrombotic ischemic stroke [107, 108]. Nevertheless, existing research recognizes the potential of D-dimer level measurements in acute stroke to conclude the underlying disease mechanism based on the extent of the value increase [106, 109, 110].

Patients with a cardioembolic cerebral vessel occlusion, most likely caused by atrial fibrillation [103, 111, 112], demonstrated higher D-dimer levels compared to cases with lacunar ischemic events [106]. This is most likely due to the structural differences between fibrin-rich thrombi of cardiac origin and platelet-rich thrombi encountered in cases of arterial blood vessel occlusions [106]. Based on the

assumption of a thrombotic small vessel occlusion as the pathophysiological correlate of lacunar ischemic stroke, the small-scale arterial thrombus formation might not be able to create measurable D-dimer level increases [106].

It is noteworthy that D-dimer levels were also significantly elevated in cases of transient cerebral ischemia (i.e., TIA) within the first week after the event as well as 1 and 3 months after the episode [113]. Spontaneous intracerebral hemorrhage may also trigger an early systemic D-dimer elevation [114]. The exact biochemical mechanisms of how a local derangement of the hemostatic equilibrium may lead to a measurable systemic response are unclear [114]. D-dimer levels were also reported to independently predict clinical progression in patients with ischemic stroke within the further course [104, 115].

A vivid example of an established area of application of D-dimer measurements in daily clinical routine is the diagnosis of cerebral venous sinus thrombosis [116]. Cerebral venous thrombosis is a relatively rare but, especially in younger individuals, considerable cause of cerebral infarction or hemorrhage, but may also mimic the clinical picture of stroke itself [116–118]. Rapid diagnosis is crucial to initiate appropriate therapeutic measures involving anticoagulation [116]. Normal D-dimer levels preclude cerebral venous thrombosis with high reliability whereas elevated levels are not reliable enough to establish the diagnosis due to a lack of specificity [116, 119–121].

The underlying cause for the observed elevations in acute ischemic stroke has not been definitively clarified. D-dimer elevations in ischemic stroke may be due to a secondary inflammatory response, potentially depending on the extent of affected brain tissue, or caused by a local dysregulation of the coagulation system [104, 108]. Interestingly, D-dimer itself is suspected of being able to promote inflammatory processes in the context of progressive ischemic stroke [104].

A potential concern regarding the informative value of D-dimer and other elements of the coagulation cascade is that a variety of these markers (e.g., fibrinogen, plasminogen) also represent acute-phase reactants [122, 123]. A multitude of acute and chronic inflammatory conditions, local (e.g., traumatic) or systemic (e.g., infectious, neoplastic), can trigger the release of these markers that might persist elevated prolonged over several days depending on the stimulus [30, 122]. Particularly noteworthy in this respect is that about 5% of patients with embolic stroke of undetermined source show occult malignancies [124]. Conversely, the incidence of stroke among patients with cancer is remarkably high with almost 15%, exceeding that of the general population by far [125]. Elevated blood D-dimer levels and multiple lesions in different vascular territories demonstrated the potential to predict the presence of occult malignancies in patients with ischemic stroke with unknown origin [126]. The pathogenetic relationship between undetected cancer and ischemic stroke has not been fully elucidated but a hypercoagulable state is considered as one of the underlying key mechanisms [124]. Reports addressing the temporal association between both entities vary, with some describing stroke as a phenomenon encountered more frequently in terminal stages of cancer, and others emphasizing the role of ischemic stroke as an early indicator for malignant primary disease [124, 125, 127].

8.3.5.3 Interleukin-6 (IL-6)

IL-6 is a cytokine and is known as a nonspecific marker of inflammation [128]. Within the central nervous system, IL-6 mediates a range of other effects such as demyelination and astrogliosis [129]. There is evidence that, although stroke is often accompanied by infections, cerebral ischemia itself may trigger an inflammatory response and may, therefore, lead to the initiation of an acute-phase reaction [128, 130–132]:

First, the extent of the IL-6 elevation in the blood correlates with the infarct size in the early phase after ischemic stroke [130, 131, 133]. Second, cerebrospinal fluid levels of IL-6 surpass the blood levels in patients with ischemic stroke [130]. Maximum serum values of IL-6 are described to occur within the first 10 days after symptom onset in patients with acute stroke [131]. Third, the IL-6 peak serum concentrations after ischemic stroke correlate with the stroke severity measured by the NIHSS score [131, 133].

Thus, elevations of C-reactive protein, tumor necrosis factor, plasma cortisol, and IL-6 have been demonstrated in the peripheral blood in cohorts of patients with ischemic stroke [128, 130, 131, 134, 135].

In nonvalvular atrial fibrillation, there is evidence for another chronological relationship considering inflammation not as a consequence of cerebral ischemia but as a potential contributing factor to thromboembolism, as it may contribute to a prothrombotic milieu, potentially increasing the risk of thrombogenesis [136]. Although elevated acute-phase proteins in the context of atrial fibrillation may be due to an underlying vascular disease, an abnormal inflammatory condition is believed to be a major contributing factor in the thrombogenesis in the context of nonvalvular atrial fibrillation [136]. This hypothesis is also supported by the observation that patients with a cardioembolic etiology of acute ischemic stroke show significantly higher median blood levels of IL-6 compared to patients with lacunar infarcts [132].

8.3.5.4 Serum Neurofilament Light Chain (SNfL)

SNfL is a neurofilament subunit and represents an essential structural element of the axonal cytoskeleton [13, 137–139]. Neurofilaments are released into the peripheral blood and cerebrospinal fluid in the course of axonal injury [13]. This might be due to a large variety of underlying acute or chronic neurological conditions such as subarachnoid hemorrhage, traumatic brain injury, multiple sclerosis, and normal pressure hydrocephalus [13, 138, 140].

However, serum neurofilament levels in patients with acute ischemic stroke and TIAs with symptom onset within the last 24 h were associated with the clinical severity (measured by the NIHSS score) on admission and the diagnosis of TIAs [13]. Based on its anatomical location, SNfL has proven to be indicative of subcortical axonal damage and constitutes a marker for the severity of white matter lesions [137]. Maximum concentration levels in the cerebrospinal fluid are reached within several days after neuronal damage [141].

A relevant cause of stroke among the 18–50 age group, accounting for approximately 20% of strokes, is cervical artery dissection [142, 143]. The exact pathophysiology has not yet been fully elucidated but hypertension, cervical trauma, and

systemic infections are some of the numerous described risk factors [142–144]. As headache and neck pain are the most frequent initial symptoms in cases of cervical artery dissection, cerebrovascular infarction as the first clinical manifestation is observed in not more than approximately one-third of cases [144]. Interestingly, the risk of consecutive ischemic stroke due to cervical artery dissection generally seems to be relatively low, whereby the majority of strokes seem to occur within the first 2 weeks after diagnosis [144]. It is worth noting that the time of treatment initiation in cases of cervical artery dissection seems to play an important role in the prevention of consecutive cerebral infarction. A study including patients with traumatic cervical artery dissection demonstrated that more than 50% of untreated patients presenting without stroke developed stroke subsequently, whereas the stroke rate could be reduced to under 5% with early treatment [144]. Recent evidence suggests that SNfL levels are higher in patients with cervical artery dissection presenting with ischemic stroke compared to patients with TIA and local symptoms [145]. Moreover, SNfL levels are associated with clinical stroke severity (measured by NIHSS score) in this cohort [145]. These findings are in line with those of a more recent study that demonstrated an association between SNfL admission levels and clinical severity on admission in patients with acute ischemic stroke and TIA [13]. In this cohort, the control group showed lower SNfL serum levels than patients with acute cerebrovascular events (i.e., acute ischemic stroke or TIA) [13].

8.3.5.5 Fibrillin-1

Fibrillin-1 is a glycoprotein and a structural component of the vascular wall [12]. The potential clinical relevance in the context of arterial dissections becomes evident since fibrillin-1 gene mutations can cause Marfan syndrome [12, 146]. In turn, hospitalized individuals with Marfan syndrome demonstrate a significantly higher rate of carotid dissections and cerebral aneurysms compared to controls, underlining its physiological function [12, 146].

In young and middle-aged adults dissections of the internal carotid and the vertebral artery account for up to 25% of ischemic strokes [147, 148]. The pathophysiological explanation for the measurable elevations of fibrillin-1 in the blood due to spontaneous acute cerebrocervical arterial dissections is related to suspected subintimal arterial injury as the starting point [12, 147]. Due to the subsequently emerging intramural hematoma, it is hypothesized that fibrillin-1 is released from within the vascular wall due to the rupture of its elastic fibers [12, 147]. Moreover, higher fibrillin-1 blood levels were associated with radiologically more severe cerebrocervical dissections [149]. However, no differences regarding fibrillin-1 elevations have been reported between intra- and extracranial arterial dissections [12].

To date, there have been no detailed investigations of fibrillin-1 serum levels in patients with other vascular comorbidities, such as atherosclerosis or temporal course of its blood concentrations, respectively [12]. Although fibrillin-1 elevations in the blood were described in aortic dissections, it is not yet clear why elevations of fibrillin-1 were also detectable in healthy controls, calling for further research regarding the biological distribution patterns within different types of tissue [12].

8.3.6 Limitations of Biomarkers

The most evident limitations when it comes to biomarker-based stroke diagnosis, at least from a clinical point of view, certainly derive from the complexity of "real" patients out of any standardized study criteria. In clinical routine, physicians frequently encounter stroke mimics and patients with multiple comorbidities including vascular diseases. How certain is it that detected elevations of certain biomarkers are attributable to an acute cerebrovascular event? The currently available data is sobering: different forms of brain tissue damage can potentially lead to elevated biomarker levels [7]. Moreover, biomarkers used for diagnostic purposes might also be released from extracerebral tissue [18].

Another fundamental problem is the heterogeneity of stroke itself with different and complex, yet not fully understood, pathophysiological relationships [7, 14]. Biomarkers are unlikely to differentiate between small and large vessel occlusions or to provide information about the exact location or the tissue at risk, all of which are essential information for guiding therapeutic decisions [17]. Given the pathophysiological alterations in the context of stroke, the blood-brain barrier may hinder or distort the transition of released biomarkers from the brain tissue into the systemic circulation, depending on its functional capacity [7, 19]. Furthermore, frequently encountered restrictions of renal or liver function could interfere with test results and potentially influence the temporal course of biomarker candidates in the blood [70].

A prerequisite for the use of diagnostic biomarkers in clinical practice is the standardization of the measurement techniques and threshold values to enhance comparability. To this date, the different measurement methods used in prior studies still represent a potential confounding factor in the interpretation of the current data [14]. Prospective studies with mixed populations are needed to validate the results of preliminary studies under real-life conditions.

8.3.7 Outlook: The Future of Biomarkers

Given the large quantity of potential diagnostic biomarkers that are subject of current research and the specific field of application of each biomarker candidate with its unique limitations, the development of diagnostic biomarker panels may be sensible to improve the accuracy for the detection of acute stroke [19].

8.4 Conclusion

The past 30 years have seen rapid advances in neuroradiological imaging techniques, especially MRI, which lead to improvements regarding the early, reliable diagnosis of cerebral ischemia and timely treatment initiation. During the last decade, considerable literature around the theme of biomarkers in the context of acute stroke diagnosis has emerged. Although raised public awareness for the

time-critical clinical picture of stroke and advances in its treatment has led to the establishment of centers specialized in the management of those patients, the heterogeneity and diversity of its clinical presentation with possible subtle initial symptoms still pose a challenge in everyday routine. To address this issue, a multitude of biomarker candidates targeting specific steps of the pathophysiological cascade were studied. Due to the complexity of the underlying and not yet fully understood mechanisms, numerous mimicking conditions, and various comorbid conditions, every marker reflects only a partial aspect of the whole disease picture and is unlikely to provide sufficient information to guide therapeutic decisions. Yet, there still is a need for further diagnostic strategies in a significant proportion of cases where the etiology of ischemic stroke remains unclear after a completed search for potential sources of embolism.

A point-of-care blood marker differentiating an ischemic from hemorrhagic stroke can inform—in the prehospital setting—the decision for or against thrombolysis, e.g., complementing prehospital brain imaging.

A blood-borne biomarker approach might be able to yield valuable information to guide further secondary prophylaxis and provide clarity on the issue to the patient with reliable statements regarding the risk of recurrence. As this chapter has demonstrated, every single biomarker candidate has its unique limitations and potential fields of application. To a large extent, this is because it reflects only a small element of the pathophysiological cascade and therefore only provides limited conclusiveness. To facilitate the distinction between cerebrovascular events and mimicking conditions and accelerate the preclinical management, a multi-biomarker panel combining different strengths and target points seems to be the most promising and practical way.

Nevertheless, before the admission for clinical use several obstacles must be overcome: first, the standardization of the measurement techniques as the basis for the comparability of results; second, the composition of a biomarker set with sufficient discriminative capacity between cerebrovascular events and mimicking conditions; and third, biomarker sets must be validated in a large international cohort with a wide range of comorbidities.

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