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BLOOD BIOMARKERS FOR STROKE DIAGNOSIS AND MANAGEMENT

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Abstract

Biomarkers are objective indicators used to assess normal or pathological processes, evaluate responses to treatment and predict outcomes. Many blood biomarkers already guide decision-making in clinical practice. In stroke, the number of candidate biomarkers is constantly increasing. These biomarkers include proteins, ribonucleic acids, lipids or metabolites. Although biomarkers have the potential to improve the diagnosis and the management of patients with stroke, there is currently no marker that has demonstrated sufficient sensitivity, specificity, rapidity, precision, and cost-effectiveness to be used in the routine management of stroke, thus highlighting the need for additional work. A better standardization of clinical, laboratory and statistical procedures between centers is indispensable to optimize biomarker performance. This review focuses on blood biomarkers that have shown promise for translation into clinical practice and describes some newly reported markers that could add to routine stroke care. Avenues for the discovery of new stroke biomarkers and future research are discussed. The description of the biomarkers is organized according to their expected application in clinical practice: diagnosis, treatment decision, and outcome prediction.

Keywords

stroke; biomarker; genomics; proteomics; diagnosis; management

INTRODUCTION

Biomarkers are objective indicators used to assess normal or pathological processes, evaluate responses to medical interventions, and predict outcomes (Atkinson et al. 2001).

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COMPLIANCE WITH ETHICAL STANDARDS

This review is based solely on previously published articles from ethically approved studies. The work did not involve human or animal experiments and did not require the collection of new data. Therefore, no ethical approval or consent was required. The manuscript has not been submitted elsewhere for publication in part or in full. All authors have approved the final version of the manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

They can refer to molecules present in body fluids (blood, cerebrospinal fluids, urine) but also to physical measurements on tissues (e.g. imaging, electrophysiology). Molecular biomarkers include proteins, metabolites, lipids, and ribonucleic acids (RNA) (Table 1) (O.Y. Bang 2017; Jickling and Sharp 2011; Whiteley et al. 2012a). They can be used alone or in combination (panels, scores or indices) to improve their diagnostic accuracy or their capacity to estimate disease risk or clinical outcome (Jickling and Sharp 2015). Several blood biomarkers are used to aid clinical decisions. For example, high-sensitive cardiac troponin T guides the diagnosis of myocardial infarction (Twerenbold et al. 2017), D-dimers are informative for the diagnosis of pulmonary embolism (Crawford et al. 2016), plasma creatinine is used to assess and monitor kidney function, antibodies targeting acetylcholine receptors help to diagnose myasthenia gravis (Lalive 2011), B-type natriuretic peptide (BNP) is used to assess heart failure, and C-reactive protein levels reflect the response to antibiotic therapy in bacterial infection (Bruns et al. 2008).

There is currently no blood biomarker used for the diagnosis of stroke. This is in part because the characteristics required are challenging including high sensitivity and specificity in a heterogenous disorder and need for a very rapid turnaround. (Jickling and Sharp 2011; Katan and Elkind 2018; Makris et al. 2018). Several reviews have summarized biomarkers studied to date in stroke (Jickling and Sharp 2011, 2015; Katan and Elkind 2018; Makris et al. 2018; Whiteley et al. 2012a; Whiteley et al. 2009a; Whiteley et al. 2008; Whiteley et al. 2012b; Glushakova et al. 2016; Ng et al. 2017; Foerch et al. 2009; Sharp and Jickling 2013; El Husseini and Laskowitz 2010; Simats et al. 2016; Li and Wang 2016; Kernagis and Laskowitz 2012). The current review does not intend to be an exhaustive description of stroke biomarkers. It is focused on blood biomarkers that show promise for translation into clinical practice and describe newly reported markers that could add to routine stroke care. Avenues for the discovery of new biomarkers and future research are discussed. The description of the biomarkers is organized according to their applications in clinical practice: diagnosis, treatment decisions, and outcome prediction. Abbreviations used are listed in Box 1.

I. BIOMARKERS FOR STROKE DIAGNOSIS

Clinicians are often faced with diagnostic challenges in the diagnosis and management of stroke. A diagnostic test for stroke is needed not only to confidently identify stroke mimics that explain more than 40% of cases presenting with an acute neurological deficit (Briard et al. 2018), but also to aid in the distinction between hemorrhagic and ischemic stroke in circumstances where access to brain imaging is limited. Early identification of patients with acute ischemic stroke is important because revascularization therapies are time-sensitive, currently limited to 4.5 hours for intravenous thrombolysis (Hacke et al. 2008; Balami et al. 2013), and up to 24 hours for endovascular thrombectomy (Nogueira et al. 2018)s. Another important challenge in stroke diagnosis is determining stroke etiology which remains cryptogenic in as many as one third of patients even after a comprehensive workup (Yaghi et al. 2017a; Saver 2016). Moreover, the pathophysiological processes involved in brain damage and repair in the context of human stroke remain poorly understood, limiting the design of adjunctive drug therapies to improve the recovery process. Several molecules are being evaluated as blood biomarkers for stroke diagnosis (Table 2).

1) Distinction between acute stroke, healthy controls and stroke mimics

Many blood proteins have the potential to distinguish stroke from disorders mimicking stroke or healthy controls, notably antibodies against the NR2A/NR2B subunits of the N-Methyl-D-Aspartate (NMDA) receptor (Dambinova et al. 2003), neuron specific enolase – NSE (Wunderlich et al. 2006), heart-type fatty acid binding protein – HFABP (Zimmermann-Ivol et al. 2004), Parkinson disease protein 7 – PARK7, and nucleoside diphosphate kinase A – NDKA (Allard et al. 2005). However, none of these protein biomarkers has made it to the clinical setting because they either showed suboptimal sensitivity and specificity in studies with small sample size and were not independently validated or because the interpretation of their performance was limited by selection or classification biases (Whiteley et al. 2008). As an example, PARK 7 (or protein deglycase-1), a redox-sensitive molecular chaperone measured by enzyme-linked immunosorbent assay, was shown to discriminate stroke from controls with 85% sensitivity and 97% specificity in a multi-center retrospective observational study that included 622 patients with stroke or transient ischemic attack and 165 controls. The diagnostic cut-off used was 1.55 μg/L (Allard et al. 2005). These promising results have not been robustly replicated to establish the benefit of measuring PARK7 in patients with suspected acute stroke in the emergency setting.

In a prospective study of 172 strokes and 133 controls, glycogen phosphorylase isoenzyme BB was found to discriminate stroke from controls with 93% sensitivity and specificity when measured within 12 hours of onset (cut-off of 7.0 ng/mL) (Park et al. 2018). Glycogen phosphorylase breaks down glycogen into glucose-1-phosphate to provide the needed metabolic energy. It is not specific for brain injuries as its plasma concentration also increases in acute coronary syndromes (Bozkurt et al. 2011) which were excluded using troponin T screening. Serum apolipoprotein A1 unique peptide (APOA1-UP) was also shown to discriminate acute ischemic stroke patients from controls with a sensitivity of 91% and a specificity of 97% in a sample of 94 ischemic strokes and 37 controls (Zhao et al. 2016). Platelet basic protein identified by mass-spectrometry seems to adequately discriminate patients with transient ischemic attacks from healthy controls. The results obtained on a sample of 20 TIAs, 15 minor strokes and 12 controls (migraine, seizures) need to be confirmed on larger cohorts (George et al. 2015). Another study using mass-spectrometry showed that a set of 30 proteins related to inflammation, coagulation, atrial fibrillation and neurovascular unit injury improved discrimination between strokes ($n = 20$) and controls ($n = 20$) compared to a model based on age alone ($p < 0.001$, cross-validated area under the ROC curve = 0.93 vs. 0.78) (Penn et al. 2018).

Researchers have also attempted to combine protein biomarkers into panels to improve their diagnostic properties. A panel of four biomarkers including serum calcium binding protein B – S100B (glial activation), von Willebrand Factor – vWF (thrombosis), Matrix Metalloproteinase 9 – MMP9, and vascular cell adhesion molecule – VCAM (inflammation) was shown to discriminate stroke from controls with 90% sensitivity and specificity (Lynch et al. 2004). In the STROKE-CHIP study ($n = 1308$), none of the 21 biomarkers tested showed sufficient accuracy to differentiate between real strokes and stroke mimics and between ischemic and hemorrhagic strokes in the hyperacute phase (Bustamante et al.

2017a). A logistic regression model including the patients' demographics and cardiovascular risk factors outperformed the model including biomarkers only, for the differentiation between ischemic stroke and ICH. The 21-biomarker panel did not include glial-specific markers such as the glial fibrillary acid protein (GFAP) which is currently the most robust biomarker of ICH (discussed below).

Transcriptional changes induced by the interaction between white blood cells and various cellular (damaged brain cells, platelets, blood clot) and humoral factors (cytokines, hormones) before or immediately after a stroke could also provide a molecular signature of stroke (Sharp and Jickling 2013; Sharp et al. 2011). These transcriptional changes could be observed either at the level of messenger RNAs (mRNAs or coding RNAs) or at the level of non-coding RNAs. To date, only mRNAs, microRNAs (miRNAs), and long non-coding RNAs (lncRNAs) have been studied as potential diagnostic biomarkers for stroke. The first study of mRNA expression in acute stroke was conducted in rat models of ischemic stroke, intracerebral hemorrhage, status epilepticus, hypoxia and hypoglycemia. Whole genome microarray was used to assess mRNA expression in leukocytes isolated within 24 hours after the index event. The study demonstrated that many mRNAs are differentially expressed in the various conditions explored but an accurate distinction of each specific condition from the others could not be done using a single mRNA. The study of a gene expression profile (a group or panel of genes) was indispensable to fully characterize each type of brain injury (Tang et al. 2001).

Using an 18-gene panel, a subsequent human study confirmed that the assessment of mRNA expression profile in peripheral blood mononuclear cells (PBMC) isolated at various time points after ischemic stroke (3, 5 and 24 hours) could discriminate acute strokes (45 samples) from controls (15 samples) with a sensitivity and a specificity greater than 85% (Tang et al. 2006). However, the genes differentially expressed in humans after an ischemic stroke were different from those reported in rats, meaning that only human studies are appropriate for subsequent transcriptomics studies of human stroke (Sharp and Jickling 2013). Therefore, a larger validation study was performed including 70 stroke patients (199 samples) and 107 controls (17 with acute myocardial infarction, 52 with various cardiovascular risk factors, 38 healthy individuals). The same 18-gene panel was used to explore mRNA expression in whole blood and had a sensitivity of 93.5% and a specificity of 89.5% for stroke diagnosis (Stamova et al. 2010). In further clinical studies, differential mRNA expression also displayed 100% sensitivity and specificity for the discrimination of patients with transient ischemic attacks from controls with a similar profile of cardiovascular risk factors. The genes differentially expressed were associated with inflammation and platelet or prothrombin activation (Zhan et al. 2011; Jickling et al. 2012).

Considering that miRNAs have a direct influence on mRNA translation (Im and Kenny 2012), it is expected that the modifications of mRNA expression observed in stroke patients would also be reflected at the level of miRNA expression. Indeed, it has been shown that miR-122, miR-148a, let-7i, miR-19a, miR-320d, and miR-4429 are decreased while miR-363 and miR-487b are increased in patients with acute stroke when compared to controls with a similar profile of cardiovascular risk factors (Jickling et al. 2014a). These miRNAs were predicted to regulate various aspects of the inflammatory and coagulation

responses in stroke. Changes in the miRNA machinery might even precede the modifications of mRNA expression. Further research is needed to refine our understanding of the role of miRNAs (both intracellular or extracellular) in stroke.

Long non-coding RNAs have also been explored as potential diagnostic biomarkers for stroke. Wang and collaborators have reported that the expression levels of the lncRNA Zinc Finger Antisense 1 (ZFAS1) had a sensitivity of 89.4% for discriminating patients with stroke due to large artery atherosclerosis from healthy subjects but with only 48% specificity (J. Wang et al. 2018). In an analysis of whole-blood RNA samples from 133 patients with ischemic stroke and 133 controls matched for vascular risk factors, 299 lncRNAs and 97 lncRNAs were differentially expressed between stroke patients and controls in males and females, respectively. There was proximity between the differentially expressed lncRNAs and some putative stroke-risk loci, including lipoprotein, lipoprotein(a)-like 2, ABO (transferase A, α1-3-N-acetylgalactosaminyltransferase; transferase B, α1-3-galactosyltransferase) blood group, prostaglandin 12 synthase, and α-adducins (Dykstra-Aiello et al. 2016).

2) Distinction between ischemic stroke and intracerebral hemorrhage

Distinguishing ischemic from hemorrhagic stroke is important as it guides therapeutic decisions. Patients with ischemic stroke benefit from intravenous thrombolysis, which is contraindicated in hemorrhagic stroke. Currently, a plain CT scan of the head is used to identify hemorrhagic stroke. This requires patients to be transported to a CT-equipped hospital which can delay the treatment. Studies have explored the use of biomarkers to quickly rule out an intracerebral hemorrhage (ICH). Such biomarkers could be useful in remote regions where transport to nearest CT scanner could take hours.

Glial fibrillary acid protein (GFAP) is a leading candidate to identify hemorrhagic stroke. GFAP is a brain-specific intermediate filament protein maintaining astroglial cell structure (Eng et al. 2000). It is only found at very low concentrations in the plasma of healthy individuals because it is not actively secreted from cells (Missler et al. 1999). However, an immediate destruction of glial cells, as is the case in ICH, causes a release of great amounts of GFAP and other glial proteins in the bloodstream within minutes. Considering that necrotic cell death and cell lysis can be delayed in ischemic stroke, the difference in GFAP release kinetics between hemorrhagic and ischemic stroke creates a diagnostic window (Brunkhorst et al. 2010). In the BE FAST 1 and 2 trials, the sensitivity-specificity of GFAP to distinguish hemorrhagic and ischemic stroke was 84.2%-96.3% and 77.8%-94.2% at a threshold of 0.29 μg/mL and 0.03 μg/mL, respectively (Foerch et al. 2012; Luger et al. 2017). The ability of GFAP to discriminate hemorrhagic from ischemic stroke has been confirmed by subsequent studies using different cut-points (Katsanos et al. 2017; Xiong et al. 2015) and in a meta-analysis (Perry et al. 2018). Unfortunately, its diagnostic performance varies from one cohort to the other and is influenced by the delay between symptom onset and sample collection, the nature of the specimen used (serum or plasma), the volume of the hematoma, the severity of the stroke, the measurement method, and eventually the ethnicity (Michal Rozanski and Audebert 2018). Also, when compared to CT

scan, the sensitivity of GFAP does not seem to be high enough for it to serve as a stand-alone test to decide whether initiating intravenous thrombolysis is safe or not.

Further studies have not clearly improved the diagnostic performance of GFAP by combining it with various other biomarkers, notably retinol-binding protein 4 (RBP4) (Llombart et al. 2016), anti-NMDA (Stanca et al. 2015), and ubiquitin carboxyl-terminal hydroxylase-L1 (Ren et al. 2016). However, no study has investigated the combination with S100B, another glial-specific protein expressed by mammalian astrocytes that discriminates ischemic stroke from intracerebral hemorrhage with a sensitivity and specificity of 95.7% and 70.4%, respectively, at a cut-point of 67 pg/mL (Zhou et al. 2016). A panel combining glial-specific and neuron-specific biomarkers might be useful to investigate in the acute stroke setting.

In a study of mRNA expression in 99 whole-blood samples from patients with ischemic strokes (n = 33), ICH (n = 33) and vascular risk factors-matched controls (n = 33), a panel of 107 differentially expressed transcripts related to T-cell receptors function could differentiate ICH from ischemic strokes and controls (Stamova et al. 2018). Further transcriptomic work is needed to better understand its potential as a biomarker to rapidly distinguish ischemic from hemorrhagic stroke.

3) Identification of stroke etiology

Stroke is a heterogenous disorder with multiple underlying etiologies. In hemorrhagic stroke, hypertension accounts for 50-70% of cases. Other etiologies include cerebral amyloid angiopathy, vascular malformations, brain neoplasm, and disorders of coagulation (Ariesen et al. 2003; de Oliveira Manoel et al. 2016). In ischemic stroke, etiologies include cardioembolism, large vessel atherosclerosis (LAA), small vessel disease, or other determined cause (e.g. dissection, mitochondrial disorder, genetic mutation) (Adams et al. 1993). Often, no clear cause of stroke can be identified despite extensive investigation, resulting in over 30% of patients having unclear or cryptogenic cause of stroke. Furthermore, multiple potential etiologies can exist in the same patient leaving uncertainty as to the exact cause. This is highlighted by the causative stroke classification system. In lacunar stroke, clinicians rely on indirect features to ascribe etiology (eg. infarct size and location) without clear methods to image the underlying small vessel pathology. Biomarkers could potentially improve stroke etiology assignment (Table 3).

For cardioembolic stroke, natriuretic peptides have been studied. There are three types of natriuretic peptides: atrial natriuretic peptide (ANP) synthesized mainly in the heart atria, B-type natriuretic peptide (BNP) synthesized mainly by the heart ventricles, and C-type natriuretic peptide (CNP) synthesized by the central nervous system and vascular tissues. ANP and BNP exist as pro-hormones that are cleaved into N-terminal inactive fragments (NT-proANP, NT-proBNP) and biologically active hormones (ANP, BNP) before the release into bloodstream (Steadman et al. 2010). The plasma concentration of the inactive fragments can be measured by immunoassays using antibodies targeting epitopes on their N-terminal end or their mid-region. The mid-regional epitopes are more stable to degradation by exoproteases than the N-terminal ones and may therefore allow a more precise estimation of the serum concentration of proANP or proBNP (Khan et al. 2008). In a prospective cohort

including 362 consecutively enrolled patients with ischemic stroke (36% cardioembolic), midregional-proANP (MR-proANP) had a sensitivity of 71% and a specificity of 60.3% for identifying cardioembolic stroke at a cut-point of 180 pg/mL (Katan et al. 2010). NT-proBNP and D-dimers have also shown good performance for the identification of cardioembolic strokes (Llombart et al. 2015; Montaner et al. 2008) and the discrimination of patients that benefit the most from anticoagulation with warfarin as compared to aspirin (Longstreth et al. 2013). A systematic review found that NT-proBNP has a summary sensitivity of 55% and a summary specificity of 93% for distinguishing cardioembolic from non-cardioembolic strokes (Bai et al. 2018). These discriminative properties are currently used in the ARCADIA trial (Atrial cardiopathy and antithrombotic drugs in prevention after cryptogenic stroke – NCT03192215), a multicenter, biomarker-driven, randomized, double-blinded, phase III trial comparing apixaban and aspirin in participants who have evidence of atrial cardiopathy and a recent stroke of unknown cause (Kamel et al. 2018). Other potential protein biomarkers of cardioembolic stroke include von Willebrand factor, tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interleukin 1 beta (IL1β). However, their diagnostic properties have not been described in detail (Tuttolomondo et al. 2009; Sato et al. 2006).

Transcriptomics studies have also described biomarkers for the etiologic classification of stroke. By analyzing changes in mRNA expression in 76 patients with ischemic stroke (194 samples), a 40-gene panel could discriminate cardioembolic from large vessel atherosclerotic stroke with more than 95% sensitivity and specificity within the first 24 hours of stroke onset (Jickling et al. 2010). A separate 37-gene panel was able to distinguish atrial fibrillation from non-atrial fibrillation cardioembolic strokes with a sensitivity and a specificity both greater than 90%. A functional analysis of the genes highlighted differences in the inflammatory profile observed in the various stroke subtypes (Jickling et al. 2010). After defining the gene expression profile of lacunar strokes (Jickling et al. 2011), the profiles of mRNA expressed were applied to 131 cryptogenic strokes patients classified as having a small deep infarct/possibly lacunar (n = 32) or a non-small deep infarct/likely embolic (n = 99). A 41-gene panel predicted lacunar stroke in 15 of the 32 small deep infarcts. The 40-gene panel was then applied to the remaining 116 embolic strokes of undetermined significance/ESUS and predicted 76 to be cardioembolic, 24 to be LAA, and 16 to remain of unclear etiology. These results suggest that up to 50% of patients diagnosed with cryptogenic stroke may have a cardioembolic source and a subset of patients in this group might benefit from anticoagulation. The NAVIGATE-ESUS trial showed no difference between aspirin and anticoagulation with rivaroxaban for the prevention of stroke recurrence in patients with an ESUS (Hart et al. 2018). Whether patients could be pre-selected by cardioembolic stroke biomarker before randomization remains unclear. Non-coding RNAs have also been associated with cardioembolic stroke. A set of 15 miRNAs were differentially expressed in 16 patients with cardioembolic stroke compared to controls (Modak et al. 2018).

Biomarkers of lacunar and LAA strokes have also been described. When compared to controls, stroke patients with LAA stroke have higher levels of C-reactive protein (CRP) (Suwanwela et al. 2006), fibrinogen (Alvarez-Perez et al. 2011), P-selectin or CD62P (Yip et al. 2006), adiponectin (O. Y. Bang et al. 2007), intercellular adhesion molecule 1 (ICAM-1)

(Mocco et al. 2001), and lipoprotein-associated phospholipase A2 (Delgado et al. 2012; Katan et al. 2014). ICAM-1 is also increased in symptomatic versus asymptomatic carotid plaques collected post-endarterectomy (DeGraba et al. 1998). ICAM-1 is not specific to large vessel atherosclerosis as it is increased in other stroke subtypes and other diseases (Hassan et al. 2003). Various other markers of endothelial dysfunction (homocysteine, vWF), coagulation/fibrinolysis (D-dimer, plasminogen activator inhibitor – PAI), and inflammation (CRP, IL-6, TNF-α) have also been associated with lacunar stroke (higher levels compared to non-stroke) (Wiseman et al. 2014).

Plasma levels of fibrillin-1 discriminate strokes due to carotid dissection (n = 99) from stroke of other causes (n = 115) and healthy controls (n = 20) with a 78% sensitivity and an 80% specificity (Zhu et al. 2018). Thus, plasma fibrillin-1 could aid in the diagnosis of stroke due to dissection in situations where there is high level of clinical suspicion, but conventional neurovascular imaging is inconclusive or not affordable.

II. BIOMARKERS FOR ACUTE STROKE TREATMENT

Once the diagnosis of stroke is made, the appropriate treatment must be administered in a timely manner to ensure the greatest benefits for patients and to avoid complications. For ICH, treatment options include reversal of anticoagulation if required, control of blood pressure, treatment of increased intracranial pressure, respiratory support if required, and supportive care and monitoring to prevent complications such as infection, seizure, hyperglycemia and metabolic derangements (Poli et al. 2017; Anderson et al. 2017; Dastur and Yu 2017). In ischemic stroke, acute treatments include intravenous administration of tissue plasminogen activator (tPA or alteplase) and endovascular thrombectomy (EVT) (Rothwell 2018). Treatment algorithms are becoming complex with the need to consider various clinical, imaging and biological parameters, notably the time of symptom onset (Balami et al. 2013), the infarct size/volume in relation to that of the penumbra (Nogueira et al. 2018; Thomalla et al. 2018; Albers et al. 2018), and the risk of hemorrhagic transformation (Jickling et al. 2014b). Various biomarkers have been explored to refine the estimation of these parameters and deliver patient-specific treatment recommendations (Table 4).

1) Estimation of the time of stroke onset and the volume of the ischemic penumbra

In acute ischemic stroke, patient selection for endovascular therapy (EVT) utilizes advanced imaging to identify regions of salvageable brain (ischemic penumbra) in comparison to the size of permanently infarcted tissue (Albers et al. 2018; Nogueira et al. 2018; Thomalla et al. 2018). Although perfusion and vascular imaging is important for the triage of acute stroke patients, it is not always readily available in all care centers. A biomarker could complement acute stroke imaging in the selection of patients for reperfusion therapy.

To date, there is no validated blood biomarker to estimate the time of stroke onset and find if a penumbra is still present in human acute ischemic stroke. Most attempts to define the molecular characteristics of the ischemic penumbra have been performed on animal brains (rodents and monkeys) and have reported increased levels of various proteins, cytokines and metabolites (lactate, glutamate, heat shock proteins such as HSP70, neuregulin, IL-1 and

IL-6, TNF-α, hypoxia inducible factor-1/HIF-1, chemokine stromal-derived factor-1/SDF-1/CXCL12, prostacyclin synthase/PGIS) or upregulation of early inducible genes (e.g. c-fos and c-jun) and anti-apoptotic genes (e.g. Bcl-2 and Bcl-xl) (Castellanos et al. 2006; Sharp et al. 2000). Only one study attempted to validate some of the reported protein biomarkers of ischemic penumbra in human stroke. In 226 adults with acute hemispheric ischemic stroke (median onset to enrolment time: 3.6 hours), including 61 with clinical-diffusion mismatch (CDM), serum interleukin-10 ≥ 23pg/mL and glutamate ≥ 130 μmol/L predicted CDM with a sensitivity of 96% and a specificity of 98%. Patients with CDM also had higher levels of IL-10, TNF-α and lower levels of NSE, IL-6, and active matrix metalloproteinase-9 (MMP-9) (Rodriguez-Yanez et al. 2011). However, the authors did not comment on the performance of the biomarker for discriminating between different estimated sizes of penumbra (small, medium or large CDM defined by a combination of admission NIHSS and lesion volume on diffusion-weighted imaging). Such distinction is important because the cost-benefit and/or the risk-benefit ratios might sometimes be against the administration of recanalization therapy in patients with small CDMs. In the DEFUSE-3 trial evaluating the benefit of EVT performed 6 to 16 hours after stroke onset, patients were only enrolled if they had a penumbra to infarct volume ratio of 1.8 or greater, with a penumbra volume > 15 mL and a core volume < 70 mL (Albers et al. 2018). Further studies are needed to refine the molecular characterization of the ischemic penumbra in human acute stroke as this could pave the way for the optimization of patients triage in the acute setting or the design of therapeutic intervention aimed at extending the therapeutic window by improving neuronal tolerance to ischemia.

2) Prediction of recanalization following intravenous thrombolysis

The rates of arterial recanalization within the first 2 hours following tPA administration are generally < 35% and depend on the location (proximal versus distal), length and composition of the thrombus (Thiebaut et al. 2018). In patients with proximal internal carotid artery, basilar artery or carotid T occlusions, the rate of recanalization could be as low as 4% (Bhatia et al. 2010). Patients with a thrombus longer than 8 mm or with a higher proportion of platelets also have lower rates of recanalization (Riedel et al. 2011; Denorme et al. 2016). Biomarkers to predict recanalization could inform the design of adjuvant therapies to improve the efficacy of tPA in areas where EVT is not readily available or when EVT is not indicated (distal clots with low NIHSS at presentation and high pretreatment modified Rankin scale - mRS) (Powers et al. 2018).

As an example, lower levels α_2-antiplasmin, and thrombin-activatable fibrinolysis inhibitor (TAFI) have been associated with successful recanalization (Marti-Fabregas et al. 2005). A study of acute stroke in mice has demonstrated that the administration of a diabody targeting PAI-1 and TAFI improves the efficacy of tPA without increasing the risk of hemorrhagic transformation (Wyseure et al. 2015). Another TAFI inhibitor is currently being evaluated in a multicenter randomized double-blind placebo-controlled phase 1b/2 trial (NCT02586233) aiming to recruit 130 patients with acute stroke presenting beyond 4.5 hours of onset and therefore not eligible for tPA (Thiebaut et al. 2018). Plasma levels of plasminogen activator inhibitor 1 (PAI-1) > 34 ng/mL have also been shown to predict proximal middle cerebral artery (MCA) recanalization resistance with a sensitivity of 84.6% and a specificity of 70%

(Ribo et al. 2004a). These results could be explained by the inhibitory effect of PAI-1 on tPA that guided the design of tenecteplase (TNK). The latter is a genetically modified tPA with increased fibrin specificity and resistance to PAI-1. TNK has a longer half-life allowing a single bolus administration at a lower dose (0.25 mg/kg, maximum 25 mg) (Keyt et al. 1994). In the EXTEND-IA TNK trial (NCT02388061), recanalization rates were twice as high in the group receiving TNK (22%, n = 101) than in the group receiving tPA (10%, n = 101). The patients receiving tenecteplase also had better 90-days functional outcome with similar rates (1%) of hemorrhagic transformation (Campbell et al. 2018).

More recently, a study recruiting 108 tPA-treated acute ischemic stroke patients demonstrated that higher plasma levels of ADAMTS13 (A Disintegrin And Metalloproteinase with ThromboSpondin type-1 motif, member 13) were associated with successful recanalization assessed by the Thrombolysis In Brain Ischemia (TIBI) flow grading system using transcranial Doppler. A cut-off of 75% predicted recanalization 2 hours after tPA treatment with 69% sensitivity and 55% specificity (Bustamante et al. 2018). The administration of ADAMTS13 has shown promise as a standalone therapy in mouse models of stroke related to arterial platelet-rich thrombi that are tPA-resistant (Denorme et al. 2016). Further studies should inform on the possibility to use this molecule alone or in combination with alteplase in human acute stroke.

3) Prediction of hemorrhagic transformation in ischemic stroke

Hemorrhagic transformation (HT) is a feared complication of reperfusion therapy. It occurs when blood extravasates into the brain parenchyma across a disrupted cerebral vessel. Depending on the severity and the type, HT is observed in 3-45% of patients with acute ischemic stroke (Balami et al. 2011; Jaillard et al. 1999). Cases of HT can be divided into asymptomatic versus symptomatic according to a set of clinical and imaging criteria. In the European Cooperative Acute Stroke Study, a symptomatic HT was defined by a neurological deterioration within the first 36 hours of stroke onset associated with a greater than 4 points increase of the NIHSS score (Yaghi et al. 2017b). The administration of tPA leads to a 10-fold increase in the rate of symptomatic hemorrhagic transformation (Brott et al. 1997). Many factors and clinical scores to predict the risk of HT have been reported, including stroke severity, administration of tPA or antithrombotics, hyperglycemia, hypertension, and cerebral white matter disease (Jickling et al. 2014b).

Several protein and transcriptomics biomarkers to predict the occurrence of HT in ischemic stroke have been described. Plasma levels of MMP-9 ≥ 140 ng/mL, cellular fibronectin (c-Fn) ≥3.6 μg/mL and serum levels of S100B ≥ 11.89 pg/mL, neuron specific enolase (NSE) ≥ 24.05 μg /mL, and vascular endothelial growth factor < 177.43 pg/mL predict HT with a sensitivity-specificity of 87%-90% (Castellanos et al. 2003), 100%-96% (Castellanos et al. 2004), 92%-48%, 24%-95%, 53%-82% (Kazmierski et al. 2012), respectively. When combining levels of PAI-1 < 21.4 ng/mL and TAFI > 180%, symptomatic HT was predicted with 75% sensitivity and 98% specificity (Ribo et al. 2004b). An mRNA expression panel comprising 6 genes (SMAD4, INPP5D, VEGI, AREG, MCFD2, and MARCH7) measured within 1.5 hour of stroke onset could identify patients that developed tPA-related HT at 24 hours with 80% sensitivity and 70.2% specificity (Jickling et al. 2013).

The biomarkers associated with risk of HT could inform the development of therapies to prevent HT despite the complexity of the underlying pathophysiology. For example, lower levels of PAI-1 are associated higher rates of recanalization (Ribo et al. 2004a) and higher rates of HT (Ribo et al. 2004b). This means that enhancing the activity of PAI-1 may decrease the risk of HT while reducing the effect of tPA if administered concurrently. Minocycline, an inhibitor of MMP-9, reduces rates of HT in animal stroke models and has shown similar effects in human stroke (MINOS trial) (Switzer et al. 2011; Jickling et al. 2014b). Confirmatory data on the neuroprotective effects of minocycline are awaited from ongoing trials (e.g. NCT03320018). Finally, there are currently four major clinical trials aiming to determine the optimal time to start anticoagulation in patients with acute ischemic stroke: ELAN (NCT03148457; Switzerland), OPTIMAS (EudraCT, 2018-003859-38; UK), TIMING (NCT02961348; Sweden), and START (NCT03021928; USA) (Seiffge et al. 2018). Whether a blood biomarker could help stratify risk of HT and guide timing of anticoagulation warrants study.

III. BIOMARKERS FOR STROKE PROGNOSIS

Predicting outcome is important to guide treatment and communicate with patients and their families regarding the expected effects of a stroke. Biomarkers offer the potential to predict prognosis in stroke, including patient response to treatment, development of complications, and long-term functional outcomes.

1) Prediction of early complications

Patients with acute stroke can suffer a wide range of complications in the hours following the onset of symptoms, including hemorrhagic transformation (discussed above), malignant cerebral edema, infarct growth with early neurological deterioration (END), and infection (e.g. pneumonia, urinary tract infection).

Approximately 10-20% of patients with complete large MCA infarcts develop a malignant cerebral edema (Balami et al. 2011). Decompressive hemicraniectomy (DHC), when performed early (< 48 hours), can reduce mortality by 50% (Vahedi et al. 2007; Powers et al. 2018). Early treatment is associated with improved outcomes (Dasenbrock et al. 2017). Studies of biomarkers to aid in the selection of candidates for DHC are scarce and generally included a small number of participants. For instance, plasma levels of S100B > 1.03 μg/L predicted a malignant course of infarction in acute MCA occlusion with 94% sensitivity and 83% specificity when measured 24 hours after stroke onset in a sample of 51 stroke (Foerch et al. 2004). Plasma levels of c-Fn > 16.6 μg/mL on admission also predicted the development of fatal malignant MCA infarction with 90% sensitivity and 100% specificity in a sample of 40 patients (Serena et al. 2005). These studies require replication.

An average of one-third of acute stroke patients experience an early neurological deterioration (END) which means a worsening of their neurological status within the first 72 hours following symptom onset (Haapaniemi and Tatlisumak 2009). The causes of early neurological deterioration are variable, including infarct growth, recurrent stroke, and infection. Identifying biomarkers to predict END could help clinicians to refine patients' selection for specific management. In a study of 197 patients with acute hemispheric

infarction (<12 hours), plasma glutamate > 200 μmol/L on admission was the most powerful and independent predictor of infarct growth on DWI (Castellanos et al. 2008). Glutamate release in the extracellular space in the context of ischemic stroke may cause infarct growth by activating the neuronal nitric oxide synthase pathway leading to the generation of toxic free radical and by inducing a spreading depolarization in the peri-infarct tissue thus increasing the metabolic demand in the context of reduced oxygen supply. This leads to the accumulation of lactate and free radicals causing protein denaturation, inflammation and ultimately cell death if the recovery machinery (heat shock proteins and neuregulin) fails to restore cell function (Castellanos et al. 2006). Inflammatory markers have also been associated with END, notably plasma ferritin > 275 ng/mL (sensitivity of 93% and specificity of 80%) in a study with 100 participants (Davalos et al. 2000), TNF-α >14 pg/mL, ICAM-1 > 208 pg/mL (Castellanos et al. 2002).

Chest and urinary infections are the most common medical complications in stroke, occurring 13 – 45% of patients (Kumar et al. 2010). In a recent systematic review, standardized CRP at 24–48 hours was independently associated with infection (OR 1.93-30.41 depending on the model) (Bustamante et al. 2017b).

2) Prediction of short and long-term outcome

Several biomarkers have been associated with short- and long-term clinical outcome after stroke (Table 5) but most of them have not improved the prediction capacities of clinical variables. Some of these biomarkers include neuroglial proteins such as S100B and HFABP (S. Y. Park et al. 2013a; S. Y. Park et al. 2013b); inflammatory markers such as IL-6, CRP, and TNF-α (Dieplinger et al. 2017; S. Y. Park et al. 2013a; Whiteley et al. 2012b); cardiac markers such as NT-proBNP and MR-proANP (Dieplinger et al. 2017; Katan et al. 2010; Whiteley et al. 2012b); and hemostatic markers such as fibrinogen and D-dimer (Haapaniemi and Tatlisumak 2009; S. Y. Park et al. 2013a). Copeptin, a neuroendocrine marker released by the hypothalamus in equimolar concentration with vasopressin, represents an exception since it could improve the prediction capacity of the NIHSS score for the 90-day functional outcome and the mortality (De Marchis et al. 2013; Katan et al. 2009).

Leptin/adiponectin ratio > 1.16 on day 1 has been associated with good 90-day functional outcome (mRS: 0-2) in 35 patients with atherothrombotic acute ischemic stroke (Carbone et al. 2015). High serum levels of mannose-binding lectin (MBL), a component of the complement activation cascade, were associated with mortality and poor 90-day functional outcome in 220 patients with acute ischemic stroke (Zhang et al. 2015). In another cohort of 220 patients with acute ischemic stroke, low levels of 25-hydroxyvitamin D (25-OHD) were associated with mortality and poor 90-day functional outcome (W. J. Tu et al. 2014). Other biomarkers of mortality and/or poor 90-day functional outcome in patients with acute ischemic stroke include high serum levels of progranulin, a multipotent growth factor (n = 216) (Xie et al. 2016); YKL-40, a glycoprotein associated with acute and chronic inflammation (n = 141, large artery atherosclerotic stroke) (Chen et al. 2017); RBP4 (n = 299, cut-point of 37.4 μg/mL, 50% sensitivity, 90% specificity) (Y. Y. Zhu et al. 2018); and neurofilament light, a neuronal scaffolding protein (n = 110) (Tiedt et al. 2018). High serum

levels of neurofilament light also correlated with infarct volume and recurrent ischemic lesions on MRI. High levels of glycated hemoglobin or HbA1c (n = 308) (H. Wang et al. 2018) and low activity of ADAMTS13 have also been associated with mortality or poor functional outcome (Sonneveld et al. 2016). All these biomarkers improved the performance of the NIHSS and other traditional risk factor models for the prediction of poor functional outcome and mortality. Further studies are needed to validate these results and clarify their clinical implications.

Many protein biomarkers have been reported for outcome prediction in patients with ICH. For example, serum fibulin-5, an extracellular matrix protein, predicted mortality (cut-off 80.7 μg/mL, sensitivity 78%, specificity 93%) and poor 90-day functional outcome (cut-off 48.5 μg/mL, sensitivity 86%, specificity 54%) in a cohort of 68 patients with acute ICH. Serum levels of fibulin-5 were also associated with disease severity (positive correlation with the NIHSS and the hematoma volume, negative correlation with the Glasgow Coma Scale) (Hu et al. 2016). Another study of 1262 patients with ICH demonstrated that admission serum levels of calcium ≤ 2.41 mmol/L could predict a poor composite 90-day prognosis (death or major disability) with 89% sensitivity and 78% specificity (L. Tu et al. 2018).

3) Risk stratification for secondary prevention

Stroke survivors are at increased risk for recurrent cerebrovascular events (Balami et al. 2011). Biomarkers may help to stratify the risk of recurrent stroke, myocardial infarction and death in patients with TIA, ischemic stroke and intracerebral hemorrhage (Table 6).

Transient ischemic attacks—In patients with TIA, the risk of recurrence ranges from 2-15% within the first 90 days (Giles and Rothwell 2007). Clinical scores such as the ABCD2 and ABCD3-I are used to predict the risk of stroke after TIA and identify high risk groups in need of urgent evaluation and therapy (Kelly et al. 2016; Knoflach et al. 2016). Biomarkers may offer the possibility to improve the accuracy of ABCD2 or ABCD3-I. For example, the neuroendocrine hormone copeptin improved the ABCD3-I capacity to predict stroke recurrence after TIA (De Marchis et al. 2014; Q. Xu et al. 2017; Katan et al. 2011). Lower plasma levels of lysophosphatidylcholine predict recurrent stroke in TIA and add to the predictive ability of the ABCD2 score (Jove et al. 2015). In the CHANCE trial, high levels of high-sensitive CRP (marker of inflammation) and soluble CD40L (marker of atherosclerotic plaque instability) were also identified as independent predictors of stroke recurrence (Li et al. 2016; Cabral et al. 2015). In the same trial, patients with increased levels of glycated albumin (GA > 15.5%, n = 1907) had similar rates of stroke recurrence whether they were in the aspirin group or in the aspirin plus clopidogrel group (Li et al. 2015).

Large artery atherosclerotic stroke—Large artery atherosclerosis is responsible for approximately 15-25% of all ischemic strokes and encompasses cervical artery atherosclerosis affecting the anterior (carotid arteries) or the posterior circulation (vertebral arteries) and stroke due to intracranial atherosclerosis (Chaturvedi and Bhattacharya 2014; Hart et al. 2014). The risk is not the same in all these subcategories and depends on the

topography of the stenosis, its grade, and the characteristics of the atherosclerotic plaque (Paraskevas et al. 2018; Markus et al. 2017).

Several protein and RNA markers of carotid plaque instability or progression have been reported. In 173 adult patients with ischemic stroke, low serum levels of omentin-1, a protein regulating vascular inflammation, were associated with the presence of instability features on carotid plaques assessed by ultrasound (ulceration and/or hypoechogenicity) (T. Xu et al. 2018). In 70 acute ischemic stroke patients, serum levels of complement complex C5b-9 were associated with plaque instability, plaque burden, and degree of carotid stenosis (Si et al. 2018). High levels of ICAM-1, high-sensitivity CRP, and lipoprotein-associated phospholipase A2 (Lp-PLA2) have also been associated with progressive or symptomatic LAA (Delgado et al. 2012; Arenillas et al. 2008; DeGraba et al. 1998; Katan et al. 2014). However, the specificity of these markers for carotid atherosclerosis is uncertain as they may also reflect the inflammatory response to brain ischemia. In the STABILITY trial, an inhibitor of Lp-PLA2 (darapladib) did not reduce the risk of ischemic stroke (Stability Investigators et al. 2014), raising questions regarding the relationship of Lp-PLA2 to the risk of ischemic stroke. The trial was designed to demonstrate the incremental effect of darapladib for the prevention of cardiovascular events in patients already receiving optimal secondary prevention therapy, including statins in 96% and coronary revascularization in 75% prior to randomization. Statins have been shown to reduce the levels of Lp-PLA2 by up to 35% (Ridker et al. 2012; White et al. 2013). Therefore, the events rate might have been lower than expected in both arms of the trial, thus limiting the probability to observe a significant effect of the adjunctive therapy.

MicroRNAs may also inform the risk of stroke in patients with carotid atherosclerosis. In 60 patients with >50% asymptomatic carotid artery stenosis, increased plasma levels of miR-199b-3p, miR-27b-3p, miR-130a-3p, miR-221-3p, and miR-24-3p were associated with progression of carotid stenosis (Dolz et al. 2017). These miRNAs play roles in inflammation, angiogenesis, endothelial and smooth muscle cell proliferation, migration, and differentiation (Urbich et al. 2008; Feinberg and Moore 2016; Maitrias et al. 2017). In another study of 170 healthy participants, increased plasma levels of miR-29c was independently associated with subclinical atherosclerosis defined as carotid intima-media thickness ≥ 0.9 mm after adjusting for age, body mass index, systolic blood pressure, total cholesterol, fasting blood-glucose, and CRP. Expression levels of miR-29c and CRP levels were positively correlated (Huang et al. 2018). Carotid intima-media thickness is a well-described and validated surrogate marker of atherosclerosis and a predictor of future cardiovascular events (Lorenz et al. 2007; Touboul et al. 2012). In a study of miRNA expression in 22 carotid plaques from patients undergoing carotid endarterectomy, higher levels of miR-200c were found in unstable carotid plaques (n = 12) defined according to findings on preoperative contrast-enhanced ultrasound and medical history (symptomatic or not, risk factors, treatment) (Magenta et al. 2018). Moreover, when analysing mRNA expression levels of selected biomarkers, miR-200c was positively correlated with biomarkers of plaque instability (MMP1, MMP9, IL-6, and monocyte chemoattractant 1 or MCP-1) and negatively correlated with biomarkers of plaque stability (zinc finger E-box binding homeobox 1 or ZEB1, endothelial nitric oxide NO synthase or eNOS, forkhead

boxO1 or FOXO1, and Sirtuin1 or SIRT1). Plasma levels of miR-200c decreased after 24 hours post-endarterectomy but returned to preoperative levels at 1 month.

Other stroke subtypes—A plasma level of high-sensitive CRP > 4.86 mg/L was associated with stroke recurrence in the Levels of Inflammatory Markers in the Treatment of Stroke (LIMITS) trial (n = 1244) (Elkind et al. 2014). In the Northern Manhattan Study (NOMAS), high plasma procalcitonin was associated with an increased risk of lacunar stroke and high plasma MR-proANP was related to an increased risk of cardioembolic stroke (Katan et al. 2016). An increase in plasma levels of free fatty acids was associated with a higher risk of stroke recurrence in patients with cardioembolic stroke (n = 105) (Choi et al. 2014). Further studies are required to confirm these finding and clarify the mechanism by which free fatty acids increase the risk of stroke in patients with cardioembolism.

An analysis of data from 2176 participants of the Stroke Prevention by Aggressive Reduction of Cholesterol Levels (SPARCL) trial demonstrated that osteopontin, neopterin, and myeloperoxidase are were independently associated with the risk of recurrent stroke and improved the prediction capacity of the Stroke Prognostic Instrument II (area under the receiver operating characteristic curve increased by 0.023, P=0.015 and continuous net reclassification improvement of 29.1%, P<0.0001) (Ganz et al. 2017; Kernan et al. 2000). Finally, low ADAMTS13 activity was associated with a higher risk of first ever ischemic stroke of any type in 5941 individuals aged 55 years or older from the Rotterdam study (Sonneveld et al. 2015).

CONCLUSIONS

Efforts to overcome the limitations of expert clinical judgement and multimodal neuroimaging in stroke medicine have resulted in the identification of several blood biomarkers that could improve the diagnosis and the management of stroke patients. These biomarkers are mainly proteins, RNA, lipids, and metabolites involved in various aspects of stroke, including brain injury and repair. For the diagnosis of stroke, the best discrimination between stroke and mimics have been observed when markers are combined in panels. GFAP is a leading candidate for the distinction between ischemic and hemorrhagic strokes and might perform better if combined with selected brain-specific markers. Likewise, to determine stroke etiology, panels of markers may also achieve sufficient sensitivity and specificity to address the heterogeneity in human stroke. For stroke treatment, serum IL-10 and glutamate may identify patients with clinical-diffusion mismatch, but further studies are needed to better define the blood biomarkers of ischemic penumbra. Several blood markers to predict HT have been described and future studies will clarify if they could inform the development of therapies to prevent HT or assist decision-making regarding the timing of anticoagulation after stroke. For stroke prognosis, plasma copeptin can add to age and NIHSS to predict functional outcome and to the ABCD2/ABCD3-I scores to predict stroke recurrence after TIA. Other markers of functional outcome include YKL-40, RBP4, and neurofilament light which require validation. Several RNA markers have been associated with atheroma plaque instability and further work is needed to determine if they could refine patient selection for carotid endarterectomy or stenting. The development of blood biomarkers to improve stroke diagnosis and management is ongoing. Additional results

regarding the role of biomarkers to aid in diagnosis, risk stratification, and treatment decisions are expected from several larger trials mentioned in this review.

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Box 1:

List of abbreviations used in the main text and tables

aHR = adjusted hazard ratio; **aOR** = adjusted odds ratio; **ADAMTS13** = A Disintegrin And Metalloproteinase with Thrombospondin type-1 motif, member 13 ; **ANP** = atrial natriuretic peptide; **APOA1-UP** = apolipoprotein A1 unique peptide; **BNP** = B-type natriuretic peptide; **CDM** = clinical diffusion mismatch; **c-Fn** = cellular fibronectin; **CRP** = C-reactive protein; **CT** = computerized tomography; **DHC** = decompressive hemicraniectomy; **END** = early neurological deterioration; **EVT** = endovascular thrombectomy; **FC** = fold change; **GFAP** = glial fibrillary acid protein; **HbA1c** = glycated hemoglobin; **HFABP** = heart-type fatty acid binding protein; **HR** = Hazard ratio; **HT** = hemorrhagic transformation; **ICAM-1** = intercellular adhesion molecule; **ICH** = intracerebral hemorrhage; **IL-10** = interleukin 10; **IL-6** = interleukin-6; **IS** = ischemic stroke; **LAA** = large artery atherosclerosis; **lncRNA** = long non-coding RNA; **Lp-PLA2** = lipoprotein-associated phospholipase A2; **MCA** = middle cerebral artery; **MBL** = mannose-binding lectin; **MMP9** = matrix metalloproteinase 9; **MiRNA** = microRNA; **MR-proANP** = mid-regional pro-atrial natriuretic peptide; **MRI** = magnetic resonance imaging; **mRS** = modified Rankin scale; **MS** = mass spectrometry; **N/A** = not applicable or not available, **NDKA** = nucleoside diphosphate kinase A; **NfL** = neurofilament light; **NMDA** = N-methyl-D-aspartate; **NIHSS** = national institutes of health stroke scale; **NSE** = neuron-specific enolase ; **NT-proBNP** = N-terminal B-type natriuretic peptide; **OR** = odds ratio; **PAI-1** = plasminogen activator inhibitor 1; **PARK7** = Parkinson disease protein 7; **PBP** = platelet basic protein; **RBP4** = retinol-binding protein 4; **RNA** = ribonucleic acid; **S100B** = serum calcium binding protein, **VCAM** = vascular cell adhesion molecule; **Se** = sensitivity; **Sp** = specificity; **TAFI** = thrombin-activatable fibrinolysis inhibitor; **TIA** = transient ischemic attack; **TNF-α** = tumor necrosis factor α; **tPA** = tissue plasminogen activator; **VCAM** = vascular cell adhesion molecule; **VEGF** = vascular endothelial growth factor; **vWF** = von Willebrand factor; **ZFAS1** = zinc finger antisense 1

Table 1:

Classification of the blood biomarkers used in stroke management

Nature	Origin	Subgroup	Classical illustrative examples	References
Proteins	Brain-specific biomarkers	Related to glial activation	Serum calcium binding protein (S100B)	(Nash et al. 2008; Foerch et al. 2007)
			Glial fibrillary acid protein (GFAP)	(Llombart et al. 2016; M. Rozanski et al. 2017)
			Myelin basic protein (MBP)	(Jauch et al. 2006)
		Related to neuronal injury	Neuron-specific enolase (NSE)	(Anand and Stead 2005; Missler et al. 1997)
			Heart fatty acid-binding protein (HFABP)	(S. Y. Park et al. 2013b; Zimmermann-Ivol et al. 2004)
			Anti-N-methyl-D-Aspartate (anti-NMDA) receptors antibodies	(Dambinova et al. 2003)
	Biomarkers not specific for the central nervous system	Related to hemostasis and endothelial dysfunction	Von Willebrand Factor (vWF) and its cleaving protein ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13)	(Bustamante et al. 2018; Sonneveld et al. 2015)
			D-Dimer	(Wiseman et al. 2014; Yoon et al. 2012)
			Fibrinogen	(del Zoppo et al. 2009; Turaj et al. 2006)
			Plasminogen activator inhibitor (PAI)	(Ribo et al. 2004b)
			Thrombomodulin	(Olivot et al. 2004; Tanne et al. 2006)
			Fibronectin	(Castellanos et al. 2007; Serena et al. 2005)
			Thrombin activatable fibrinolysis inhibitor	(Ribo et al. 2004a)
			Adhesion molecules: soluble intercellular adhesion molecule (ICAM), Vascular cell adhesion molecule (VCAM)	(Bitsch et al. 1998)
		Related to inflammation	C-reactive protein (CRP)	(Bustamante et al. 2017b; VanGilder et al. 2014)
			Matrix metalloproteinase 9 (MMP-9)	(Ramos-Fernandez et al. 2011)
			Lipoprotein-associated phospholipase A2	(Elkind et al. 2009)
			Cytokines: Interleukins (IL-6, IL-10) and tumor necrosis factor alpha (TNF-α)	(Whiteley et al. 2009b; Tuttolomondo et al. 2009)
			Plasma ferritin	(Davalos et al. 2000)
		Related to apoptosis	Caspase-3	(Rosell et al. 2008)
		Neuroendocrine markers	Copeptin	(Katan et al. 2011; Q. Xu et al. 2017)

Nature	Origin	Subgroup	Classical illustrative examples	References
			Natriuretic peptides and their precursors: atrial natriuretic peptide (ANP), midregional pro-atrial natriuretic peptide (MR-proANP), B-type natriuretic peptide (BNP), N-terminal pro-B-type natriuretic peptide (NT-proBNP).	(Katan et al. 2010; Llombart et al. 2015)
			Adiponectin	(Efstathiou et al. 2005)
RNAs	Differential expression of messenger RNA (mRNA)	40 gene panel	ADAMTSL4, AP3S2, ARHGEF12, ARHGEF5, BANK1, C16orf68, C19orf28, CD46, CHURC1, CLEC18A, COL13A1, EBF1, ENPP2, EXT2, FCRL1, FLJ40125, GRM5, GSTK1, HLA-DOA, IRF6, LHFP, LHFP, LOC284751, LRRC37A3, OOEP, P2RX5, PIK3C2B, PTPN20A, TFDP1, TMEM19, TSKS, ZNF185, ZNF254	(Jickling et al. 2010)
		41 gene panel	ALS2CR11, C18orf49, CALM1, CCDC114, CCDC78, CCL2, CCL3, CHML, FAM179A, FAM70B, FLJ13773, GBP4, GTF2H2, HLA-DQA1, HLADRB4, IL8, LAG3, LAIR2, LGR6, LRRC8B, MPZL3, OASL, PDXDC1, PROCR, PRSS23, QKI, RASEF, RUNX3, SCAND2, STK4, STX7, TGFBR3, TSEN54, TTC12, UBA7, UGCG. UTS2, VAPA	(Jickling et al. 2011)
	Differential expression of non-coding RNA	MicroRNAs (miRNA)	miR-200c	(Magenta et al. 2018)
			Let-7i	(Jickling et al. 2016)
		Long non-coding RNAs (lncRNA)	Zinc Finger Antisense 1 (ZFAS1)	(J. Wang et al. 2018)
Lipids			Oxidized low-density lipoprotein	(Uno et al. 2003)
			Free fatty acids	(Choi et al. 2014)
			Lysophosphatidylcholine	(Jove et al. 2015)
Metabolites			Plasma glutamate	(Castellanos et al. 2008)
			Nitric oxide	(Castillo et al. 2000)

Table 2:

Biomarkers used for the differential diagnosis of stroke

Clinical purpose	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	Se (%)	Sp (%)	Ref.
Stroke versus no stroke (Mimics, Controls)	Protein	Anti-NMDA	360 samples from 105 strokes and 255 controls	≥ 2 μg/L	< 3h	97	98	(Dambinova et al. 2003)
		NSE	66 strokes	N/A	< 3h	N/A	N/A	(Wunderlich et al. 2006)
		HFABP	22 strokes, 22 controls, 22 acute myocardial infarctions	OD > 0.531	< 24h	68.2	100	(Zimmermann-Ivol et al. 2004)
		NDKA	622 strokes, 165 controls	≥ 2.55 μg/L	< 24h	73	97	(Allard et al. 2005)
		PARK7	622 strokes, 165 controls	≥ 1.55M μg/L	< 24h	85	97	(Allard et al. 2005)
		Glycogen phosphorylase isoenzyme BB	172 strokes, 133 controls	7.0 ng/mL	< 12h	93	93	(Park et al. 2018)
		Serum APOA1-UP	94 strokes, 37 controls	Ratio APOA1-UP to reference protein > 1.80	N/A	91	97	(Zhao et al. 2016)
		PBP	35 TIA/minor IS, 12 controls	1500 ng/mL	< 48h	91	57	(George et al. 2015)
	4-protein panel	S100B, vWF, MM9, VCAM	65 strokes, 157 controls	N/A	< 6h	90	90	(Lynch et al. 2004)
	18-gene panel (mRNA)	ARG1, BCL6, CA4, CKAP4, ETS-2, HIST2H2AA, HOX1.11, F5, FPR1, LY96, MMP-9, NPL, PYGL, RNASE2, S100A9, S100A12, S100P, SLC16A6	70 strokes, 107 controls	>|1.5| FC	< 3h	93.5	89.5	(Stamova et al. 2010)
	MicroRNAs	Downregulated: miR-122, miR-148a, let-7i, miR-19a, miR-320d, and miR-4429 / Upregulated: miR-363 and miR-487b are increased	24 strokes, 24 controls	>|1.2| FC	< 72h	N/A	N/A	(Jickling et al. 2014a)
	Long non-coding RNA	ZFAS1	176 strokes, 111 controls	N/A	< 48h	89.4	48	(J. Wang et al. 2018)
Ischemic versus Hemorrhagic stroke	Protein	GFAP	205 patients (39 ICH, 163 IS, 3 mimics)	≥ 0.29 μg/L	< 24h	84.2	96.3	(Foerch et al. 2012)
		S100B	46 ICH, 71 IS	≥ 67 pg/mL	< 6h	95.7	70.4	(Zhou et al. 2016)

Table 3:

Biomarkers used for the etiologic diagnosis of stroke

Clinical purpose or question	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	Se (%)	Sp (%)	Ref.
Cardioembolic stroke	Protein	MR-proANP	362 IS	≥ 180 pg/mL	< 72h	71	60.3	(Katan et al. 2010)
		NT-proBNP	1840 IS (meta-analysis)	N/A	N/A	55	93	(Bai et al. 2018)
Cardioembolic versus LAA stroke	40-gene panel	ADAMTSL4, AP3S2, ARHGEF12, ARHGEF5, BANK1, C16orf68, C19orf28, CD46, CHURC1, CLEC18A, COL13A1, EBF1, ENPP2, EXT2, FCRL1, FLJ40125, GRM5, GSTK1, HLA-DOA, IRF6, LHFP, LHFP, LOC284751, LRRC37A3, OOEP, P2RX5, PIK3C2B, PTPN20A, TFDP1, TMEM19, TSKS, ZNF185, ZNF254	76 cryptogenic strokes (194 samples)	>|1.2| FC	< 3h	95	95	(Jickling et al. 2010)
Lacunar versus non-lacunar stroke	41-gene panel	ALS2CR11, C18orf49, CALM1, CCDC114, CCDC78, CCL2, CCL3, CHML, FAM179A, FAM70B, FLJ13773, GBP4, GTF2H2, HLA-DQA1, HLADRB4, IL8, LAG3, LAIR2, LGR6, LRRC8B, MPZL3, OASL, PDXDC1, PROCR, PRSS23, QKI, RASEF, RUNX3, SCAND2, STK4, STX7, TGFBR3, TSEN54, TTC12, UBA7, UGCG. UTS2, VAPA	184 cryptogenic strokes	>|1.5| FC	< 72h	> 90	> 90	(Jickling et al. 2011)
LAA stroke	Proteins (tested in independent studies)	CRP, fibrinogen, P-selectin (CD62P), adiponectin, ICAM-1, Lp-PLA2	Variable sample sizes	N/A	N/A	N/A	N/A	(Alvarez-Perez et al. 2011; O. Y. Bang et al. 2007; Delgado et al. 2012; Katan et al. 2014; Mocco et al. 2001; Suwanwela et al. 2006; Yip et al. 2006)
Lacunar stroke	Proteins (tested in independent studies)	Homocysteine, vWF, D-dimer, PAI-1, CRP, IL-6, TNF-α	Variable sample sizes	N/A	N/A	N/A	N/A	(Wiseman et al. 2014)
Cervical artery dissection	Protein	Fibrillin-1	214 IS (99 with cervical artery dissection), 20 controls	≥ 88.5 ng/mL	N/A	78	80	(Zhu et al. 2018)

Table 4:

Biomarkers used to guide stroke treatment

Clinical purpose	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	Se (%)	Sp (%)	Ref.
Estimation of the penumbral volume	Panel (Protein/cytokine, metabolite/ neurotransmitter)	IL-10, Glutamate	226 IS (61 with clinical-diffusion mismatch)	IL-10 ≥ 23 pg/mL, glutamate ≥130 μmol/L	< 12h	96	98	(Rodriguez-Yanez et al. 2011)
Prediction of recanalization after thrombolysis	Protein	PAI-1	44 IS (proximal MCA occlusion)	≥ 34 ng/mL	< 3h	84.6	70	(Ribo et al. 2004a)
		ADAMTS13	108 IS	Activity ≥ 75%	< 4.5h	69	55	(Bustamante et al. 2018)
Prediction of spontaneous HT (no tPA)	Protein	MMP9	250 IS	≥ 140 ng/mL	< 15h	87	90	(Castellanos et al. 2003)
		S100B	458 IS	≥ 11.89 pg/mL	< 48h	92	48	(Kazmierski et al. 2012)
		NSE		≥ 24.05 μg/mL	< 48h	24	95	
		VEGF		< 177.43 pg/mL	< 48h	53	82	
Prediction of HT after thrombolysis	Protein	c-Fn	87 strokes	≥ 3.6 μg/L	< 4.5h	100	96	(Castellanos et al. 2004)
	2-protein panel	PAI-1 and TAFI	77 strokes	PAI-1 < 21.4 ng/mL, TAFI activity > 180%	< 3h	75	98	(Ribo et al. 2004b)
	6-gene panel (mRNA)	SMAD4, INPP5D, VEGI, AREG, MARCH7, MCFD2	44 strokes	>|1.2| FC	< 3h	80	70.2	(Jickling et al. 2013)

Table 5:

Biomarkers to predict early complications and the 90-day functional outcome [a]

Outcome	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	Se (%)	Sp (%)	Ref.
Malignant cerebral edema in MCA occlusion	Protein	S100B	51 strokes	≥ 1.03 μg/L	< 24h	94	83	(Foerch et al. 2004)
		c-Fn	75 IS	≥ 16.6 μg/mL	< 9h	90	100	(Serena et al. 2005)
Early neurological deterioration or infarct growth	Neurotransmitter	Glutamate	197 IS	> 200 μmol/L	< 12h	aOR = 89.7	95%CI: 19.8 – 406.6	(Castellanos et al. 2008)
	Protein	Ferritin	100 IS	> 275 ng/mL	< 16h	93	80	(Davalos et al. 2000)
		ICAM-1	113 lacunar strokes	> 208 pg/mL	< 24h	aOR = 315	95%CI = 17 - 5748	(Castellanos et al. 2002)
	Protein (cytokine)	TNF-α	113 lacunar strokes	> 14 pg/mL	< 24h	aOR = 511	95% CI: 17 – 4937	(Castellanos et al. 2002)
Infection	Protein	CRP	697 IS (meta-analysis)	N/A (> fourth quartile)	24-48h	aOR = 3.21	95%CI: 1.93–5.32	(Bruns et al. 2008)
Poor functional outcome at 90 days	Proteins (individually associated with poor 90-day functional outcome but did not improve the prediction capacity of clinical parameters or NIHSS)	S100B, IL-6, CRP, TNF-α, NT-proBNP, MR-proANP, fibrinogen, D-dimers	Variable sample sizes	N/A	N/A	N/A	N/A	(S. Y. Park et al. 2013a; S. Y. Park et al. 2013b; Dieplinger et al. 2017;, Haapaniemi and Tatlisumak 2009; Katan et al. 2010; Whiteley et al. 2012b)
	Proteins (individually associated with poor 90-day functional outcome with improvement of the prediction capacity of the NIHSS)	MBL, progranulin, YKL-40, NfL, HbA1c, ADAMTS13	Variable sample sizes	Variable	N/A	N/A	N/A	(Zhang et al. 2015; Xie et al. 2016; Chen et al. 2017; Tiedt et al. 2018; H. Wang et al. 2018; Sonneveld et al. 2016)
	Protein	Copeptin	359 strokes	N/A (per log unit increase)	< 72h	aOR = 4.23	95% CI: 1.61–11.15	(Katan et al. 2009)
		RBP4	299 IS	≥ 37.4 μg/mL	< 48h	50	90	(Y. Y. Zhu et al. 2018)
		Serum fibulin-5	73 ICH	≥80.7 μg/mL	< 72h	78	96	(Hu et al. 2016)
	Vitamin	25-hydroxyvitaminD	220 IS	N/A (per unit increase)	< 12h	OR = 0.79	95% CI: 0.73–0.85	(W. J. Tu et al. 2014)
	Electrolyte	Calcium	1262 ICH	≤ 241 mmol/L	< 12h	89	78	(L. Tu et al. 2018)

[a] Wherever appropriate, the sensitivity (Se) and specificity (Sp) have been replaced by the adjusted odds ratio (aOR) and the corresponding 95% confidence interval (CI), respectively.

Table 6:

Biomarkers used to predict stroke recurrence, plaque instability and response to antithrombotic treatments

Outcome to predict	Type	Biomarker	Sample size	Cut-off	First sample collection (after onset)	aHR	95% CI	Ref.
Stroke recurrence	Protein	Copeptin [a]	107 TIAs	≥ 9 pmol/L	< 72h	Se = 80%	Sp = 76%	(Katan et al. 2011)
		High-sensitive CRP	3044 TIA/minor IS	> 3 mg/L	< 36h	1.5	95%CI: 1.1 – 2.0	(Li et al. 2016)
		Soluble CD40L	3044 TIA/minor IS	> third tertile	< 36h	1.5	95%CI: 1.1 – 2.0	(Cabral et al. 2015)
	Proteins (tested in independent studies)	procalcitonin, osteopontin, neopterin, myeloperoxidase, ADAMTS13	Variable sample sizes	N/A	N/A	N/A	N/A	(Katan et al. 2016; Ganz et al. 2017; Sonneveld et al. 2015)
	Lipids	Free fatty acids	105 cardioembolic IS	N/A (per unit increase)	< 7 days	2.7	95%CI: 1.1 – 7.0	(Choi et al. 2014)
	Metabolite	Lysophosphatidylcholine [b]	293 TIA	< 1,14,000 MS counts	< 24h	OR = 3.5	95% CI: 1.5 – 8.9	(Jove et al. 2015)
Plaque progression or instability	Proteins (tested in independent studies)	Omentin-1, complement complex C5b-9, ICAM-1, CRP, Lp-PLA2	Variable sample sizes	N/A	N/A	N/A	N/A	(T. Xu et al. 2018; Si et al. 2018; Arenillas et al. 2008; Delgado et al. 2012; DeGraba et al. 1998; Katan et al. 2014)
	MicroRNAs	MiR-199b-3p, miR-27b-3p, miR-130a-3p, miR-221-3p, miR-24-3p, miR-29c, miR-200c	Variable sample sizes	Variable thresholds of FC	N/A	N/A	N/A	(Dolz et al. 2017; Huang et al. 2018; Magenta et al. 2018)
Response to dual antiplatelets antiplatelet therapy	Protein	Glycated albumin	1907 TIA/minor IS	> 15.5%	< 36h	0.8 (vs 0.4)	95%CI: 0.6 – 1.1 (vs 0.3 – 0.6)	(Li et al. 2015)
Benefit from anticoagulation	Protein	NT-proBNP	1028 IS	> 750 pg/mL	< 30 days	0.3 (vs 1.2)	95%CI: 0.1 – 0.8 (0.9 – 1.7)	(Longstreth et al. 2013)

[a] The adjusted hazard ratio (aHR) and the corresponding 95% confidence interval (CI) have been replaced by the sensitivity (Se) and specificity (Sp), respectively.

[b] The adjusted hazard ratio (aHR) has been replaced by the odds ratio (OR).