

Circulating Biomarkers in Long-Term Stroke Prognosis: A Scoping Review Focusing on the South African Setting

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Abstract

Cerebrovascular disease, including both ischaemic and haemorrhagic strokes, remains one of the highest causes of global morbidity and mortality. Developing nations, such as South Africa (SA), are affected disproportionately. Early identification of stroke patients at risk of poor clinical prognosis may result in improved outcomes. In addition to conventional neuroimaging, the role of predictive biomarkers has been shown to be important. Little data exist on their applicability within SA. This scoping review aimed to evaluate the currently available data pertaining to blood biomarkers that aid in the long-term prognostication of patients following stroke and its potential application in the South African setting.

This scoping review followed a 6-stage process to identify and critically review currently available literature pertaining to prognostic biomarkers in stroke.

An initial 1191 articles were identified and, following rigorous review, 41 articles were included for the purposes of the scoping review. A number of potential biomarkers were identified and grouped according to the function or origin of the marker. Although most biomarkers showed great prognostic potential, the cost and availability will likely limit their application within SA.

The burden of stroke is increasing worldwide and appears to be affecting developing countries disproportionately. Access to neuroradiological services is not readily available in all settings and the addition of biomarkers to assist in the long-term prognostication of patients following a stroke can be of great clinical value. The cost and availability of many of the reviewed biomarkers will likely hinder their use in the South African setting.

Categories: Internal Medicine, Neurology, Physical Medicine & Rehabilitation

Keywords: mortality, morbidity, biomarkers, south africa, stroke, prognosis

Introduction And Background

Cerebrovascular disease, including ischaemic and haemorrhagic strokes, remains one of the highest causes of morbidity and mortality globally, with an estimated stroke prevalence of 104.2 million people, resulting in over 6 million deaths in 2017 alone [1]. Low-to-middle income countries (LMIC), such as South Africa (SA), are disproportionately affected compared to wealthier nations [2,3]. Strokes result in an excess of 100 disability-adjusted life years (DALYs) in SA alone, with one study in rural SA showing a burden of the cost of more than R4.2-million (approximately US\$264000) in sub-district health expenditure [4,5].

Early identification, confirmation and management of suspected acute ischaemic strokes (AIS) and intracerebral haemorrhages (ICH) result in improved functional outcomes [6,7]. Neuroradiological imaging, the gold standard diagnostic test in stroke medicine, incurs significant costs, with the global computed tomography (CT) scan market alone exceeding US\$6billion in 2020 [8]. CT brain, the investigation of choice when a stroke is suspected, can cost upwards of ZAR4000 (approximately US\$280), whilst magnetic resonance imaging (MRI) can cost more than ZAR10000 (approximately US\$700). This clearly limits the wide implementation of these imaging modalities in LMIC, such as SA. The need for cheap, widely accessible diagnostic and prognostic tools such as biomarkers, which have been shown to have significant additive predictive value, is therefore evident [9].

The term biological marker, or biomarker, has been ascribed various definitions. Strimbu and Tavel and Puntmann emphasise the objectivity of measured biomarkers [10,11]. The Food and Drug Administration-National Institutes of Health (FDA-NIH) Biomarker Working Group published an updated version of their document titled BEST (Biomarkers, Endpoints, and other Tools) Resource on the 25th of January 2021.

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Various types of biomarkers are defined and discussed [12]. Of interest in the current review are prognostic biomarkers in acute strokes. A prognostic biomarker is “used to identify (the) likelihood of a clinical event, disease recurrence or progression in patients” presenting with a specific diagnosis [12].

A biomarker aiming to assist in long-term prognostication in strokes should ideally be detectable early in the disease process, widely available, easily interpreted, and must have an appropriate sensitivity to ensure false negatives are avoided. The use of biomarkers following various insults to the brain has been well described. Numerous studies and systematic reviews have been published over the years, including a study by Jickling and Sharp, who found that more than 58 biomarkers in ischaemic stroke have been described [13-19].

This scoping review aimed to determine the currently available data pertaining to blood biomarkers that aid in the long-term prognostication of patients following AIS or ICH and its application within the SA setting.

Review

Although no definition of a scoping review has been universally accepted, essential themes and purposes have been identified which are ever-expanding [20,21]. Scoping reviews aim to rapidly determine important ideas in a specific research area [22]. It assists in determining the scope of research done in its breadth, depth and nature [22,23]. Mays et al. are of the view that scoping reviews “can be undertaken as standalone projects in their own right” [24]. Extensive work has been published by Arksey and O’Malley, further strengthened by work by Levac et al. [20,22]. These authors recommend a 6-stage process that has been adapted for the purpose of this review.

Stage 1 required establishing the research question and discussion with a team of researchers with expertise in the field. The question “What is the currently available literature regarding the evidence for the use of blood biomarkers in long-term prognostication following stroke?” was decided upon. A long-term prognosis is defined as functional or clinical outcome more than 30 days following the stroke event. Identification of relevant studies (stage 2) required the determination of keywords, which, with the help of Boolean operators (AND/OR/NOT), aided in refining the search terms in multiple databases. Following the application of filters such as English language and human participants, the search resulted in the identification of literature from PubMed/Medline (228 articles), ScienceDirect (890 articles) and SciELO (16 articles). A further 57 articles were identified during the reading process.

As a result, a total of 1191 articles were identified for analysis. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart was utilised to screen these articles for selection (stage 3) and, following the application of rigorous exclusion criteria, a total of 41 articles were included for critical review (Figure 1) [25].

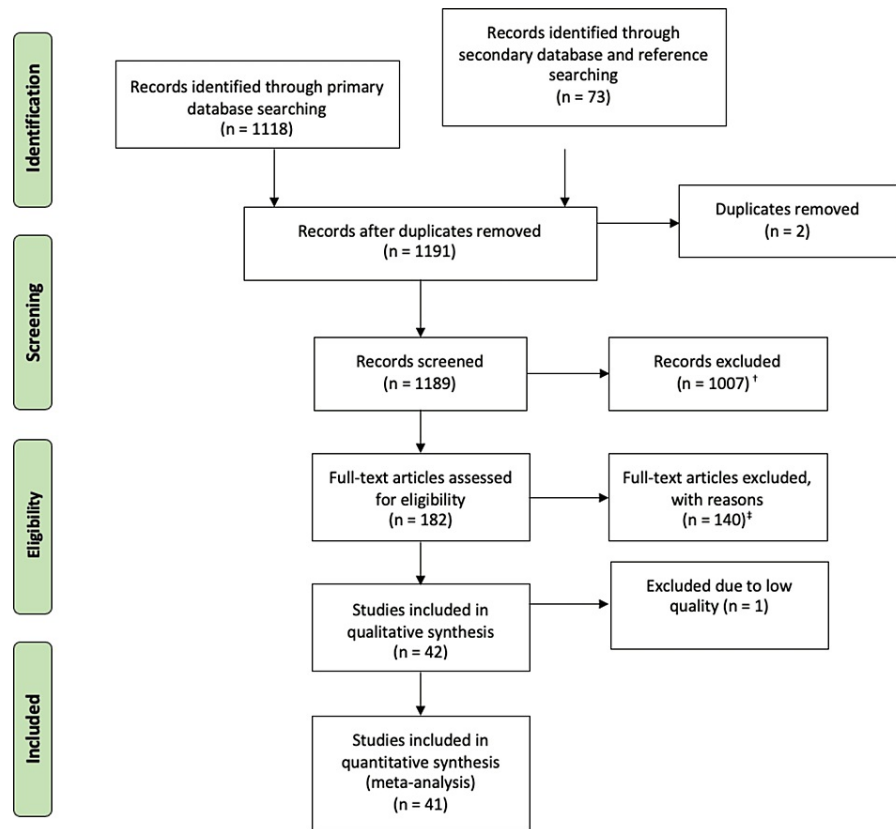


FIGURE 1: The Study Selection Process Following the PRISMA Flowchart

PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis. Original image created by the authors.

†Study/trial unrelated to research question: 583; study/trial focussing on stroke risk factors: 36; study/trial focussing on management of stroke: 24; study/trial focussing on cardiac pathologies: 283; study/trial focussing on atrial fibrillation: 35; study/trial focussing on cardiorenal syndromes: 5; study/trial focussing on coronavirus disease 2019 (COVID-19): 30; study/trial focussing on sickle cell disease: 8; study/trial focussing on artificial intelligence in disease: 3

‡Review article/meta-analysis/editorial: 34; full text not available, only abstract: 6; study on stroke risk/chronic strokes/transient ischaemic attacks: 15; study on biomarkers and mainly other diseases in stroke: 20; study on differentiating stroke types and stroke mimics: 7; study focussing on diagnosis/management/clinical decision-making: 28; study determining risk of acute outcomes: 9; study to determine pathophysiology of disease: 7; study criteria not specific: 2; animal/laboratory-based studies (validation): 11; study looking at cost associated with stroke: 1

Data were extracted and charted (stage 4) from each study following critical analysis based on the methods described by Young and Solomon [26]. Data were collated and summarised (stage 5) below, following which a conclusion was drawn (stage 6). Arksey and O’Malley recommend that “a thematic construction is used to provide an overview of the breadth of the literature” [22]. As such, following critical analysis, the 41 key articles have been summarised (Table 1) [27-67]. The nature of the articles has allowed for a categorical theme which is discussed below.

Author	Year	Stroke Type	Serum Biomarker Assessed
Biomarkers of Inflammation			
Christensen et al. [27]	2002	Ischaemic	TNFa IL-1b IL-1RA IL-6 IL-10 TNF-R1 TNF-R2
Smith et al. [28]	2004	Ischaemic	IL-6

Sotgiu et al. [29]	2006	Ischaemic	TNFa ICAM-1 IL-6
Welsh et al. [30]	2009	Ischaemic	IL-6
Smedbakken et al. [31]	2011	Ischaemic	ALCAM
Tsai et al. [32]	2014	Ischaemic	TBARS
Xu et al. [33]	2018	Haemorrhagic	Tim-3
Li et al. [34]	2020	Haemorrhagic	OPN
Acute Phase Reactants			
Di Napoli et al. [35]	2001	Ischaemic	CRP
Christensen et al. [27]	2002	Ischaemic	Ferritin
Montaner et al. [36]	2006	Ischaemic	CRP
den Hertog et al. [37]	2009	Ischaemic	CRP
Welsh et al. [30]	2009	Ischaemic	CRP
Huangfu et al. [38]	2020	Haemorrhagic	SAA
Biomarkers of Haemostasis			
Di Napoli et al. [35]	2001	Ischaemic	Fibrinogen
Nomura et al. [39]	2004	Ischaemic	TM
Jauch et al. [40]	2006	Ischaemic	TM
Carter et al. [41]	2007	Ischaemic	Fibrinogen FVIII beta-TG vWF t-PA FXIII A subunit
Welsh et al. [30]	2009	Ischaemic	d-dimer
Taylor et al. [42]	2020	Ischaemic	vWF:Ag-ADAMTS13Ac ratio
Nuclear Material			
Rainer et al. [43]	2003	All	Plasma DNA
Liang et al. [44]	2019	Ischaemic	microRNA-140-5p
Zuo et al. [45]	2020	Ischaemic	circFUND1 circPDS5B circCDC14A
Creatinine			
Carter et al. [41]	2007	Ischaemic	Creatinine
Hormones			
Denti et al. [46]	2004	Ischaemic	IGF-1
Zweifel et al. [47]	2011	Haemorrhagic	GH
Wang et al. [48]	2016	Ischaemic	Copeptin
Armbrust et al. [49]	2017	Ischaemic	IGF-1 IGFBP-3
Yang et al. [50]	2017	Ischaemic	NT-proBNP
De Marchis et al. [51]	2018	Ischaemic	MRproANP
Tu et al. [52]	2018	Ischaemic	Irisin
Arnold et al. [53]	2020	Ischaemic	MRproANP
Amino Acids, Proteins and Enzymes			
Alvarez-Sabin et al. [54]	2004	Haemorrhagic	MMP-3
Wunderlich et al. [55]	2005	Ischaemic	H-FABP B-FABP
Sotgiu et al. [29]	2006	Ischaemic	MMP

Carter et al. [41]	2007	Ischaemic	Albumin Haemoglobin
Yan et al. [56]	2016	Haemorrhagic	Galectin-3
Zhong et al. [57]	2017	Ischaemic	tHy
Zhong et al. [58]	2017	Ischaemic	MMP-9
Zeng et al. [59]	2019	Ischaemic	Galectin-3
Qian et al. [60]	2020	Ischaemic	Endostatin
Zhang et al. [61]	2020	Ischaemic	Endostatin
CNS-Specific Biomarkers			
Abraha et al. [62]	1997	All	S100B
Wunderlich et al. [63]	2006	Ischaemic	NSE Tau protein
Jauch et al. [40]	2006	Ischaemic	S100B NSE MBP
Delgado et al. [64]	2006	Haemorrhagic	S100B
Sotgiu et al. [29]	2006	Ischaemic	BDNF
García-Berrocoso et al. [65]	2013	Ischaemic	GELS DRP2 GELS/DRP2 GELS/DRP2/CYTA
Tiedt et al. [66]	2018	Ischaemic	NfL
Zhu et al. [67]	2019	Ischaemic	DDK-1

TABLE 1: Studies Included in the Scoping Review and the Biomarkers Assessed

TNF α : tumour necrosis factor alpha; IL-1b: interleukin 1 beta; IL-1RA: interleukin 1 receptor antagonist; IL-6: interleukin 6; IL-10: interleukin 10; TNF-R1: tumour necrosis factor receptor 1; TNF-R2: tumour necrosis factor receptor 2; ICAM-1: intercellular adhesion molecules 1; ALCAM: activated leukocyte adhesion molecule; TBARS: thiobarbituric acid-reactive substances; Tim-3: T-cell immunoglobulin and mucin-domain 3; OPN: osteopontin; CRP: C-reactive protein; SAA: serum amyloid A; TM: thrombomodulin; FVIII: factor eight; beta-TG: beta-thromboglobulin; vWF: von Willebrand factor; t-PA: tissue plasminogen activator; FXIII A subunit: factor thirteen A subunit; ADAMTS13Ac: activity of disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; IGF-1: insulin-like growth factor; IGFBP-3: insulin-like growth factor binding protein 3; NT-proBNP: N-terminal fragment of B-type natriuretic peptide; MRproANP: midregional pro-atrial natriuretic peptide; MMP: matrix metalloproteinase; H- and B-FABP: heart and brain type fatty acid-binding protein; tHy: total homocysteine; NSE: neuron-specific enolase; MBP: myelin-basic protein; BDNF: brain-derived neurotrophic factor; GELS: gelsolin; DRP2: dihydropyrimidinase-related protein-2; CNS: central nervous system; CYTA: cystatin A; NfL: neurofilament light chain; DDK-1: Dickkopf-1

Categories of biomarkers studied

Inflammation

As is the case in AIS, the disruption of the supply of oxygen to the brain parenchyma results in focal ischaemia (or necrosis), following which a number of biochemical and cellular changes occur [68]. Necrosis results in the release of reactive oxygen species (ROS) which promotes oxidative stress, and nucleic acids which promote cytokine and chemokine release, which in turn results in the recruitment of microglia [69]. Microglial cells play a critical role in the inflammatory cascade by upregulating the production of a number of proinflammatory chemokines and cytokines, which results in the deleterious effects following the acute insult, perpetuating the cycle [70]. Although fundamentally different in its initial insult, inflammation similarly plays an important role in secondary cellular damage following ICH [71]. These measurable cytokines and chemokines represent a potential quantitative assessment of the extent of the insult, forming the theoretical basis for their use as prognostic biomarkers.

(i) Tumour necrosis factor alpha (TNF α) and its receptors: TNF α , a cytokine produced within the central nervous system (CNS) by both neurones and glial cells, is an activity-dependent cytokine with low levels in the normal physiological state [72]. Often thought of as the prototypical proinflammatory cytokine, it has both advantageous (such as maintaining healthy myelin) and deleterious (such as inducing cellular necrosis) effects, thought to be the result of different cellular receptors [73]. Its usefulness as a prognostic biomarker has yielded contradictory results, which may reflect this pleiotropy [27,29].

(ii) Interleukins (IL): ILs, produced by a myriad of cell types throughout the body, play an imperative role in the homeostasis of inflammatory cellular function, including cellular activation, suppression, proliferation and migration [74]. Functionally, ILs may be considered pro-inflammatory or anti-inflammatory, with levels maintained in a fine balance in normal physiological states. Disruption in this homeostasis results in the deleterious effects seen following cerebral insults [75-77]. Measurement of ILs (and their direct drivers) as

tools for prognostication in strokes has resulted in mixed results [27-30,34]. Of all the markers studied, IL-6 appears to have the greatest potential of being clinically useful.

(iii) Adhesion molecules: Adhesion, leukocyte rolling and subsequent cellular transmigration of inflammatory cells are largely regulated by adhesion molecules [78]. Within the CNS, these molecules play a role in neuronal cell migration, synapse formation and inflammation [79]. The production of these molecules is rapidly upregulated following initiation of the inflammatory cascade following cerebral ischaemia [80]. Both activated leukocyte adhesion molecule (ALCAM) and intercellular adhesion molecule 1 (ICAM-1) have shown promising results as biomarkers [29,31].

(iv) T-cell immunoglobulin and mucin-domain: The Tim gene family, expressed on T-cells, B-cells and dendritic cells, underpins the complexity of immune regulation and dysregulation in a number of conditions [81]. The interplay between a number of Tim-subsets results in different immune responses to inflammation and may promote or suppress the inflammatory cascade. Tim-3 has been shown to downregulate the T helper 1 response, thereby acting as an anti-inflammatory molecule [82]. Tim-3 has been studied in ICH and has been found to be potential as both a prognostic biomarker and a potential therapeutic target [33].

(v) Thiobarbituric acid-reactive substances (TBARS): The inflammatory response following cerebral ischaemia is driven, in part, by the production of ROS [83]. Malondialdehyde, a by-product of lipid peroxidation, is produced in excess as part of the inflammatory response following cerebral ischaemia and can be measured, indirectly, by measuring TBARS [84]. Early measurement of TBARS predicts early clinical outcome as well as long-term prognosis following AIS [32].

Acute Phase Reactants (APR)

APR vary greatly in the presence of inflammation and is often a surrogate for the extent of the systemic inflammatory response to various insults [85]. APRs, including C-reactive protein (CRP) and ferritin, are known to increase following cerebral insults [86,87].

(i) C-reactive protein (CRP): CRP, first discovered in 1930, is primarily induced by the presence of IL-6 and produced by the liver [88]. Not only has CRP been found to be increased following stroke, but it has also been shown to be a predictor of new-onset strokes [89]. Results have been fairly conclusive and support its use as a prognostic biomarker following AIS [30,35,36].

(ii) Ferritin: Ferritin is the primary storage form of iron and increases in response to hepcidin [90]. Hepcidin, in turn, is thought to be an APR and has evolved to reduce iron availability, a metabolic rate-limiting step for many pathogens [91]. Ferritin, a commonly analysed laboratory parameter, is therefore thought to serve as a surrogate marker for hepcidin, and therefore inflammation. As such, authors have sought to determine the potential use of ferritin as a prognostic biomarker. Unfortunately, results have not been supportive of this [27]. This may reflect a timing issue in blood sampling due to the delay from the initiation of the inflammatory cascade, increased levels of hepcidin and subsequent increased iron storage as ferritin.

(iii) Serum amyloid A (SAA): Isolated more than half a century ago, SAA has been under much research and has been primarily identified in pathological states, with recent advances showing some role in normal lipid metabolism [92]. Predominantly synthesised in the liver, SAA acts as a pleiotropic immune modulator, with a predominantly pro-inflammatory effect [93]. A recent study by Huangfu et al. revealed the significant predictive value of SAA following ICH [38].

Mediators of Haemostasis

Numerous factors play a critical role in the maintenance of normal, laminar vascular flow to the brain, with disruption in endothelial wall integrity, flow or coagulability resulting in thrombosis [94]. Focal cerebral ischaemia is often due to local hypercoagulability and it can be postulated that the level of activity of mediators of haemostasis represents the extent of thrombosis [95]. Focal coagulopathies appear to play a central role in haematoma formation following ICH, which is directly correlated to functional outcomes [96].

(i) Pro-coagulation: Peripherally measured factors which promote thromboses, such as the von Willebrand factor, factors VIII and XIII and beta-thromboglobulin have been shown to have a significant ability to predict both mortality and morbidity following strokes [41,42]. Studies assessing fibrinogen revealed mixed results [35,41].

(ii) Anti-coagulation: Thrombomodulin, a naturally occurring anticoagulant, found on endothelial cell membranes failed to yield a clinically significant prognostic value [39,40]. Endogenous tissue plasminogen activator activity, however, appeared to predict poor outcomes well [41].

(iii) Coagulation end-products: Following fibrinolysis, d-dimer is produced as a soluble fibrin degradation

product and is detectable in the serum [97]. A paper by Welsh et al. provided evidence that d-dimer levels following AIS have a good prognostic value [30].

Nuclear Material

The nuclear material is released following cell death and can be detected using specialised equipment [98]. Primarily utilised in so-called liquid biopsies in cancer detection, free DNA and RNA material have been identified as potential markers for the extent of cerebral damage following strokes, and may therefore predict outcomes. Some studies support this claim when assessing plasma DNA and specific microRNA [43,44]. Research looking at other circular nuclear material has not supported its use as a prognostic biomarker [45].

Creatinine

Creatine, an amino acid-like compound, is predominantly produced in the liver and kidneys, but has been shown to be a key in normal cellular function within the brain [99]. Creatinine, a metabolic by-product of creatine homeostasis, has been widely studied [100,101]. Literature on its use as a prognostic biomarker following strokes is limited, however, it has been shown to be a good predictor of mortality following AIS [41].

Hormones

Various hormones have been shown to have a role in stroke risk as well as the neuroinflammatory response following a stroke [102-104]. This has provided an opportunity to assess a number of hormones as prognostic biomarkers in stroke.

(i) Growth hormone (GH)/insulin-like growth factor-1 (IGF-1) pathway: Released from the anterior pituitary somatotrophic cells GH, also known as somatotropin, fulfils a multitude of functions either directly by binding to target cells or indirectly via the action of IGF-1 [105]. Following the initiation of the inflammatory cascade, multiple factors, including GH-responsive genes (such as the rat serine inhibitor 2 locus), suppress the expression of GH and allow for it to be considered a negative APR (meaning levels reduce in the face of inflammation) [106]. Following ICH, however, activation of the hypothalamic-pituitary axis promotes the release of GH, with elevated levels being associated with poor clinical outcomes [47]. Following production in the liver, IGF-1 is bound to IGF-1 binding protein (IGFBP) and acts on IGF-1 receptors to promote cellular growth [107]. An inverse relationship exists between stroke risk and functional outcomes following strokes and IGF-1 and IGFBP-5 levels [108].

(ii) Natriuretic peptides: Natriuretic peptides consist of three structurally similar hormones which fulfil a primary cardioprotective role [109,110]. Elevated levels of both atrial- and brain natriuretic peptides have been associated with stroke risk, aetiology and prognosis [111-113]. A number of studies have confirmed the utility of natriuretic peptides as prognostic biomarkers following strokes [50,51,53].

(iii) Copeptin: Antidiuretic hormone (arginine vasopressin, ADH) is synthesised in the hypothalamus and stored within the posterior pituitary gland where it is released in response to hypovolaemia and hypernatraemia [114]. Measurement of ADH is made difficult due to various technical factors and copeptin, an amino acid glycopeptide, which has shown good potential as a biomarker in various disease states, has been shown to be an accurate surrogate marker for ADH release [115]. Elevated levels of copeptin measured shortly after symptom onset following AIS are a good predictor of both morbidity and mortality [48].

(iv) Irisin: Produced by the enzymatic cleavage of a protein found on myocyte membranes, irisin is a key hormone in the regulation of brown adipocytes [116]. Low levels of irisin are associated with sedentary lifestyles and its relationship with obesity and metabolic regulation has been suggested [117,118]. Reduced irisin levels following AIS are associated with increased psychological morbidity [52].

Amino Acids, Proteins and Enzymes

(i) Homocysteine: The methionine derived amino acid homocysteine is necessary for cellular homeostasis [119]. The association between homocysteine and atherosclerosis dates back to the late 1960s with stroke complicating hyperhomocysteinaemia [120]. In addition to its role in the pathogenesis of the cardiovascular disease, homocysteine levels have been found useful in predicting poor clinical outcomes following AIS [57].

(ii) Haemoglobin: The iron-containing protein haemoglobin is essential for intravascular oxygen transport and delivery [121,122]. Aberrant haemoglobin concentrations, both abnormally high and low, are associated with strokes in all stages of its pathophysiology [123-126]. Low haemoglobin levels are predictive of mortality following cerebral ischaemia [41].

(iii) Albumin: As the most abundant plasma protein, albumin fulfils a number of functions [127]. Acting as a negative APR, albumin has long been considered a useful tool in determining the extent of inflammation [85]. Carter et al. found that albumin is a good prognostic marker of mortality following AIS [41]. Hypoalbuminaemia may represent a poor pre-stroke physiological reserve, which increases the risk of death following a cerebral insult [128].

(iv) Fatty acid-binding protein (FABP): To date, nine FABPs have been identified and form part of the intracellular lipid-binding protein family and are involved in the binding and trafficking of intracellular hydrophobic ligands [129,130]. The heart-type FABP is predominantly found within cardiac myocytes and has been shown to be a good prognostic biomarker following both myocardial ischaemia as well as AIS, whilst the brain-type FABP has been shown to provide significant prognostic value following AIS [55,131].

(v) Galectin: The family of beta-galactoside-binding animal lectins, galectin, has been shown to be involved in a number of physiological and disease processes [132]. Some classes of galectins are predominantly expressed within the brain and are fundamental in the formation and migration of specific neuronal tissue following injury [133]. Galectin-3, a pleiotropic molecule, has in recent years been the target of a number of investigational therapeutics in a wide variety of conditions [134-136]. Its use as a potential diagnostic and prognostic biomarker in cardiovascular disease, especially, has been proven [56,59,137].

(vi) Matrix metalloproteinase (MMP): The zinc-dependent family of enzymes, MMPs, are critical in maintaining allostasis within the extracellular matrix [138]. Of the more than 20 MMPs, a number of them have become important biomarkers in a host of diseases and may prove a therapeutic target in future, including strokes [139,140]. MMP polymorphisms have been identified as an important consideration in both the pathophysiological processes and clinical outcomes following strokes [141]. MMP-9 in particular has been shown to be a key factor in the disruption of the blood-brain barrier following strokes and is associated with stroke severity [142]. Its role in prognostication following both ICH and AIS has been proven [29,54].

(vii) Endostatin: The angiogenic response following cerebral ischaemia appears to be an important defensive reaction and has a direct effect on long-term neurological recovery [143]. The mechanisms underpinning angiogenesis are complex and rely on the balance of stimulating and inhibitory factors [144]. Endostatin, a potent inhibitor of angiogenesis found in vascular walls and basement membranes, is associated with poor functional outcomes as well as mortality following AIS [60,61].

CNS-Specific Biomarkers

(i) S100B: Concentrated within glial cells, S100B functions as a calcium-binding protein with a not yet fully understood role [145]. Its clinical use as a marker of neurological disorders has been well established, with elevated levels both in the CSF and in the peripheral circulation representing active disease or neuronal damage [146,147]. S100B reliably predicts the severity of the neuronal injury, however, its utility as a prognostic biomarker in stroke has yielded mixed results [40,62,64,148].

(ii) Neuron-specific enolase (NSE): One of three isoenzymes of enolase, NSE serves a critical role in neuronal differentiation [149]. NSE has found significant utility in a number of neurological and oncological disorders [150,151]. Its clinical utility in stroke-related cerebral insults has resulted in mixed evidence from literature [40,63].

(iii) Wnt pathway: The Wnt signalling pathway is crucial to cellular regulation, including cell migration, cell polarity and neural patterning [152]. The increased expression of the Wnt antagonist, Dickkopf-1 (DKK-1), results from neurodegenerative processes and in further neurodegeneration via a complex pathway resulting in cellular death (due to the inhibition of BCL-2 expression and induction of BAX) [153]. This canonical loss of Wnt signalling results in the phosphorylation of tau protein [154]. The microtubule-associated neuronal protein, tau, is phosphorylated under normal physiological conditions, however, excessive phosphorylation results in self-aggregation resulting in tauopathies [155]. The build-up of these oligomers is associated with increased morbidity and mortality and supports the rationale for the use of both DKK-1 and tau as prognostic biomarkers [63,67].

(iv) Neurofilament light chain (NfL): Neurofilaments are particularly abundant in axons, which are dependent on NfL to maintain axonal diameter, and are detectable in pathological states due to neuronal cell death [156,157]. The high translational value of NfL has promoted its use as a prognostic biomarker in a host of neurodegenerative disorders, including multiple sclerosis [158-161]. Its utility as a prognostic biomarker in stroke has been confirmed by Tiedt et al. [66].

(v) Brain-derived neurotrophic factor (BDNF): Neuronal plasticity requires BDNF, a highly regulated molecule, which shows great variability in both health and disease [162]. It is predominantly expressed in the CNS and the gut and is involved in regulating energy metabolism and upregulating pro-survival genes, with reduced levels associated with neurodegenerative disorders [163]. The relationship between BDNF and functional outcomes following AIS has been shown [29].

(vi) Myelin-basic protein (MBP): Myelin formation within the CNS is highly dependent on the activity of MBP, and oligodendrocyte differentiation requires fine regulation of MBP expression [164]. MBP readily interacts with a host of other proteins allowing it to participate in transmission of extracellular signals [165]. Elevated central and peripheral levels of MBP are associated with cerebral damage [166]. MBP does not increase early following the onset of cerebral ischaemia and this may explain why it has not been found to be a useful biomarker in stroke [40,167].

(vii) Novel brain-derived biomarkers: Gelsolin, dihydropyrimidinase-related protein-2 and cystatin A have been identified by researchers following experimental identification in animal studies, with human post-mortem confirmation [65]. Although the function of these molecules is yet to be determined, the authors found that their presence following a stroke is associated with poor functional outcomes.

Applications in South Africa

Successful management of stroke, including diagnosis and treatment decision-making, is highly time-sensitive [168-170]. Neuroradiological services are not readily accessible to many communities in SA, notably communities that rely on public healthcare, and significant delays in accessing facilities that provide these services are experienced. This affects time-to-diagnosis and time-to-treatment which, specifically in stroke medicine, may result in a significant delay in patient care, and poorer outcomes. Biomarkers may provide an additive tool; however, limitations remain. Although some readily available biochemical tests have shown significant potential in predicting patient outcomes following stroke, cost and availability limit their use. For instance, the GH and NT-proBNP tests cost ZAR121.16 (approximately US\$8) and ZAR509.45 (approximately US\$32), respectively [171].

Some biomarkers, including SAA and IGF-1 are available, however, are not being widely utilised. These tests require specialised equipment, which is often only available at specialist centres. The cost analysis, therefore, needs to include the transport of the samples in addition to the laboratory cost, which, as in the case of IGF-1, can be nearly ZAR500 (approximately US\$32) [171]. Potential biomarkers that have been identified in diseases other than strokes, such as copeptin, DDK-1 and MRproANP, were reported in multiple articles suggesting wider applicability in strokes. These tests are not readily, if at all, available in the private and public healthcare sectors in SA. The difficulty and cost of these tests make it unlikely that they will be available anytime soon [172]. The significant disparity between LMIC and high-income countries poses a barrier to their effective implementation of novel biomarkers in the near future [173].

The delay from blood sampling to the availability of a biomarker result must also be considered. The currently available biomarkers in Uganda (an LMIC), GH and IGF, for instance, have a turnaround time of 2-3 days and 3-5 days, respectively [174]. Although its diagnostic potential is severely impacted by this delay, it might still be of prognostic value.

People within SA can access either public or private healthcare, with the former providing care from a resource-limited setting. The introduction of the National Health Insurance bill will likely result in the private healthcare sector sharing the financial burden, and the role of cost-effective adjuncts in managing patients with stroke will become ever more important.

Implications and recommendations

Cerebrovascular events, whether ischaemic or haemorrhagic in nature, result in significant morbidity and mortality worldwide [175]. Early detection and risk stratification yield improved patient outcomes [6,7]. Numerous clinical and radiological scoring systems have been introduced to determine the outcome for patients following a stroke; however, the availability of neuroradiological imaging in LMIC greatly limits its implementation. McLane et al. compared the availability of neurodiagnostic tools, such as MRI and CT, between LMIC and wealthier nations and revealed a significant disparity, “diagnostic gap” [176]. Novel approaches are required to bridge this gap.

The availability of objective, measurable biomarkers can provide accurate prognostication and identify high-risk individuals following a stroke. This is especially true in communities where access to specialised testing is limited. The advent of point-of-care testing, for markers such as creatine kinase (CK) and amino-terminal pro-peptide counterpart (NT-proBNP), has been shown to have a significantly positive impact on health outcomes in resource-limited settings and should be further researched [9,177].

Conclusions

This scoping review revealed the currently available biomarkers from published literature. Laboratory investigations range from widely utilised tests to newly identified experimental tests. The applicability in SA, based on cost and availability, varies greatly. The scoping review has limitations. First, publication bias needs to be considered. Research that has either not been submitted due to statistically insignificant results or research declined by journals cannot be assessed. Second, the reviewers are not fluent in languages other than English, limiting the review of articles published in languages such as Mandarin, Spanish, French, German or Japanese.

Biomarkers show tremendous promise in aiding clinicians in the early prognostication of patients following cerebrovascular events. This scoping review highlights the need for further research to be performed to assess new biomarkers, in terms of both clinical and laboratory accuracy and cost-effectiveness, which are readily available in the SA setting.

Additional Information

Disclosures

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