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Review

Blood biomarkers for triaging young adults and children for a suspected stroke: every minute counts

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Abstract: Early stroke diagnosis remains a big challenge in healthcare partly due to the lack of reliable diagnostic blood biomarkers, which in turn leads to increased rates of mortality and disability. Current screening methods are optimised to identify patients with a high risk of cardio-vascular disease, especially among the elderly. However, in young adults and children, these methods suffer low sensitivity and specificity and contribute to further delays in their triage and diagnosis. Accordingly, there is an urgent need to develop reliable blood biomarkers for triaging patients suspected of stroke in all age groups, especially children and young adults. This review explores some of the existing blood biomarkers, as single biomarkers, or biomarker panels, and examine their sensitivity and specificity for predicting stroke. A review was performed on PubMed and Web of Science for journal articles published in English during the period 2001 to 2021 which contained information regarding biomarkers of stroke. In this review article, we provide comparative information on the availability, clinical usefulness, and time-window periods of eight single blood biomarkers and six biomarker panels that have been used for predicting stroke in emergency situations. The outcomes of this review can be used in future research for developing more effective stroke biomarkers.

Keywords: stroke, CNS, ischaemic, haemorrhagic, biomarker, panel, young adults, children, triage, specificity, sensitivity, prediction values

1. Introduction

Stroke is the leading cause of disability and the second most common cause of death worldwide [1]. Early detection of stroke is essential for implementing timely diagnostic tests and radio-imaging as well as subsequent intervention therapies such as thrombolysis (using tissue plasminogen activator), thrombectomy, or anti-platelet/ anti-coagulant treatments [2-6]. However, early detection of stroke is still remaining elusive and it has been reported that even in many advanced hospitals only about one-third of the patients with ischaemic stroke are diagnosed early enough for a timely intervention [2].

Early screening tools, such as the Cincinnati Prehospital Stroke Scale (CPSS) or the Recognition of Stroke in the Emergency Room (ROSIER) scale, have demonstrated their values in high-risk patients, with a sensitivity between 80% and 85% [2, 7]. However, these tools are less accurate in children and young adults, who account for 10%-15% of all stroke cases [2, 8]. Given there are approximately 12 million new cases of stroke diagnosed globally each year, it is estimated that there are around 1-2 million cases per year that are not detected appropriately using the current screening tools [9]. In addition, studies have found that current screening tools have poor performance in distinguishing stroke from stroke mimics such as migraine, epilepsy, central nervous system (CNS) infections, Bell's

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palsy, and conversion disorders, with a negative predictive value of approximately 20% [2, 10].

The majority of the current screening tools for stroke are based on considering the patients' clinical signs and symptoms and demographic risk factors. The downside is that those patients who do not present with typical symptoms and those who are perceived as low risk (e.g., children and young adults) may not be consistently identified [8]. Therefore, we need alternative methods for detecting potential stroke cases which do not depend on the above-mentioned categorisations. Such screening tools would be a welcome addition to the diagnostic toolkit of clinicians at emergency departments, neurology departments, and regional hospitals, as well as paramedics.

The use of blood biomarkers plays an important role in the screening and diagnosis of some critical illnesses such as ischaemic heart disease. The inclusion of troponin into the screening/diagnostic protocols of ishaemic heart disease in early 2000s significantly improved the clinical approach to this condition, and subsequently has contributed to remarkable improvements in patient outcomes [11]. Unfortunately, this is not the case with the screening and diagnosis of stroke.

The brain is a complex tissue comprising different unique cells including various neurons and glial cells as well as an extracellular supportive matrices [12]. Therefore, in the event of a stroke where many neuronal tissues are damaged, a sudden release of CNS and/or vascular biomarkers into the peripheral blood would be expected. If such biomarkers are reliably measured in the peripheral blood specimens, then they could be used for screening or triaging purposes.

In this article, we have reviewed many currently available stroke biomarkers and assessed their potential usefulness for detection or prediction of stroke in suspected patients.

2. Materials and Methods

We performed a review of the literature published in English language from 2001 to 2021 using two online databases, PubMed and Web of Science. We used the search terms including "stroke", "diagnosis", "biomarker", "humans", "sensitivity", and "specificity". We also screened the reference lists of the extracted articles to identify articles not computed from the original search.

3. Results and Discussion

The initial database search generated 170 results. Three articles were excluded as duplicates, and after screening the titles and abstracts of the remaining we included 23 articles for this review (Figure 1).

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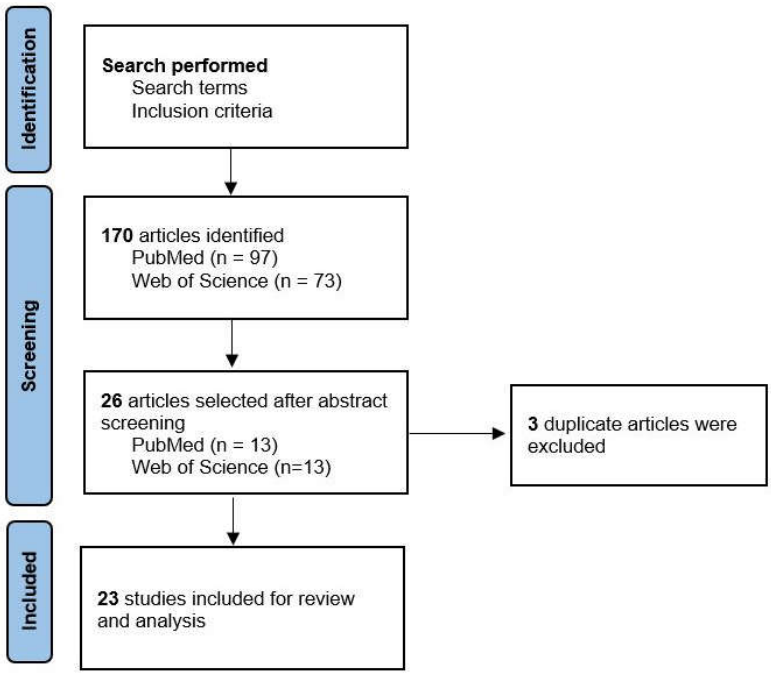

Figure 1. The literature search processes

3.1 Individual biomarkers

Over the last 20 years, many biomarkers have been studied for stroke detection, however, we still do not have a reliable biomarker which can detect stroke with such a high accuracy compared to troponin in the diagnosis of ischaemic heart disease. Nonetheless, many biomarkers have been identified whose blood levels increase following a stroke. In general, these biomarkers can be divided into a few categories based on their origins, namely: 1) the neuronal injury markers (e.g. heart-type fatty acid binding protein (H-FABP), anti-N-methyl-d-aspartic acid (anti-NMDA), Parkinson disease protein 7 (PARK7), nucleoside diphosphate kinase A (NDKA), apolipoprotein A1 unique peptide (APOA1-UP), matrix metalloproteinase-9 (MMP-9), glycogen phosphorylase isoenzyme BB (GPBB), and B-type neurotrophic growth factor (BNGF)) [13-18]; 2) the neuronal cell activation indicators (e.g. S100 calcium-binding protein B (S100B) and monocyte chemoattractant protein-1 (MCP-1)) [19, 20]; 3) the neuroinflammation indicators (e.g. eotaxin and vascular cell adhesion molecule (VCAM)) [21, 22]; 4) the endothelial dysfunction markers (e.g. D-dimer, von Willebrand factor (vWF); and 5) the neuro-endocrine markers such as B-type natriuretic peptide (BNP), and cortisol [23].

Despite the abundance of available biomarkers, only a few of them have demonstrated a sensitivity above 50% for stroke in clinical trials, which largely limits their clinical applicability [24]. In the process of this literature review, we focused on biomarkers that have undergone preliminary clinical evaluations. We identified eight individual biomarkers that have both a sensitivity and specificity of more than 50% (Figure 2).

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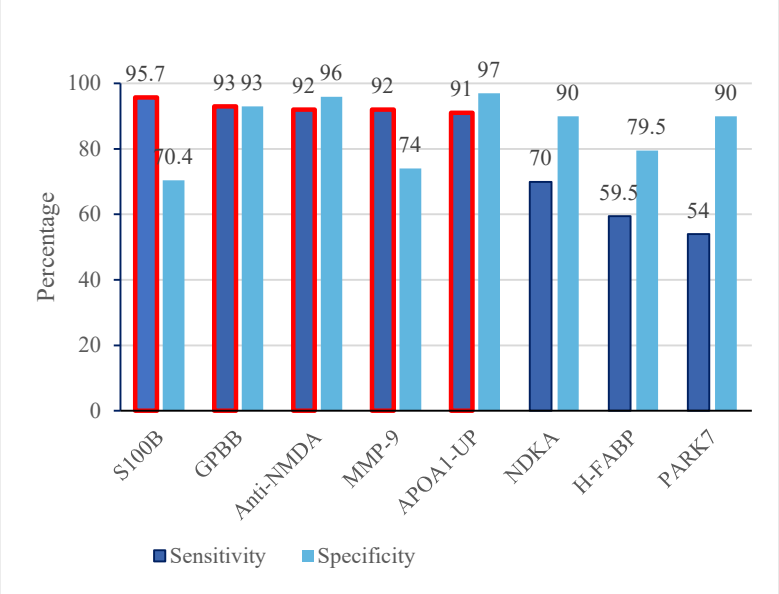

Figure 2. Single blood biomarkers for stroke. S100B, GPBB, anti-NMDA and MMP-9 all have sensitivities of >90% (highlighted in red). S100B, S100 calcium-binding protein B; GPBB, glycogen phosphorylase BB; Anti-NMDA, anti-N-methyl-d-aspartic acid; MMP-9, matrix metalloproteinase-9; APOA1-UP, apolipoprotein A1 unique peptide; NDKA, nucleoside diphosphate kinase A; H-FABP, heart-type fatty acid binding protein; PARK7, Parkinson disease protein 7.

S100B is a member of the S100 protein superfamily. It is an intracellular protein found in glial cells and Schwann cells and is released into the blood circulation following cellular activation caused by tissue damage [25, 26]. S100B has a sensitivity of 95.7% and specificity of 70.4% for stroke as well as an area under the curve (AUC) of 0.903 in differentiating between ischaemic stroke (IS) and intra-cranial haemorrhage (ICH) [19]. This biomarker is also useful in predicting the patient's short-term functional outcome after a stroke event [25]. However, the elevations of the plasma levels of this biomarker in other neurological and neuropsychological disorders such as Alzheimer's disease and schizophrenia means it is of reduced usefulness in triaging the suspected patients (i.e., a less-than-ideal specificity for stroke) [26].

GPBB is a glycogen phosphorylase isoenzyme found in the brain and heart tissues whose function is to make glucose-1-phosphate by breaking down glycogen, which helps restore the energy stores depleted during cerebral ischaemic events [17]. According to Park et al., (2018), increased plasma levels of GPBB have a sensitivity and a specificity of 93% for detecting stroke within 12 hours from the onset of the symptoms [17]. However, this study did not find any correlation between GPBB levels and the severity of the stroke, infarct volume or the clinical outcome, which suggests a less suitable position for this biomarker to be used for predicting the disease prognosis in patients with IS.

Anti-NMDA is an antibody against a proteolytic degradation product of NMDA receptor (NR2 peptide) which is released during brain tissue injury [14]. During a stroke event, the breakdown of the blood-brain barrier can release these brain antigens to the peripheral circulation which is followed by the formation of antibodies against NMDA whose levels are predictive of stroke and other adverse neurological outcomes in high-risk situations [14, 27]. In a study undertaken by Dambinova et al., in 2013 it was reported that anti-NMDA has a sensitivity of 92% and specificity of 96% for ischemic stroke when anti-NMDA levels are measurable at 3 hours post-stroke [14].

MMP-9 is a Zn^{2+}-dependent proteolytic enzyme which is released from different cells including but not limited to neutrophils and has roles in the degradation of extracellular matrix following IS and ICH [28]. Experimental studies have shown that a systemic

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inflammation during stroke causes a neutrophil infiltration of the ischemic area of the brain which eventually leads to increased plasma MMP-9 activity in patients with stroke [29]. The studies by Castellanos et al., (2007) and Kelly et al., (2008) showed that high levels of MMP-9 are predictive of blood brain barrier disruption due to blood vessel transformation after stroke [18, 30]. Accordingly, it was reported that the measurement of MMP-9 in blood samples taken within 24 hours from the onset of stroke symptoms showed a 92% sensitivity and 74% specificity for the IS [18].

APOA1-UP is a major protein component of high-density lipoprotein. It has been reported that this peptide exhibits anti-inflammatory and antioxidant effects that can be relevant to stroke. Studies have shown that levels of APOA1-UP decrease in patients with stroke and/or infection [31]. Similarly, decreased levels of this protein have a high sensitivity (91%) and specificity (97%) for stroke, nominating it as a promising independent predictor of the IS [16].

PARK7 and NDKA are released from the cerebrospinal fluid into the plasma after significant brain injury [15]. The study done by Allard et al., (2005) reported a sensitivity of 54% and a specificity of 90% for PARK7 at a cut-off level of 14.1 μg/L, when samples were taken at 3 hours after the onset of the acute stroke. Accordingly, the reported sensitivity and specificity for NDKA, at a cut-off value of 22 μg/L were 70% and 90% respectively [15].

H-FABP is a fatty-acid binding protein which is released from CNS tissues after an ischemic event into the blood. A study by Park et al., found that this protein had a sensitivity of 59.5%, specificity of 79.5%, and an AUC = 0.71 ($P < 0.001$) for identifying an ischemic stroke if the blood samples were collected after 24 hours of the stroke onset [13]. Given the long timeframe and its low sensitivity, this protein might not be a good biomarker for stroke detection.

Although many of these biomarkers seem promising in the early screening of stroke, most of the findings are hardly generalisable to larger populations due to the small sample sizes of the original studies. In addition, because medical interventions need to be performed within a short timeframe to salvage the vulnerable neuronal tissues and minimise the mortality or functional deficit, many of the suggested biomarkers do not seem to be very useful because of the relatively long time needed from the symptoms' onset until a reliably measurable change in the biomarkers' levels can detected. Some biomarkers, such as PARK7, NDKA and anti-NMDA, are released into the plasma and are detectable within the first three hours after the stroke onset, which makes them potentially promising biomarkers to be used in future studies in acute settings [15]. Unfortunately, many other biomarkers identified in this review have not yet been evaluated for their diagnostic reliability at the early stages of stroke. Table 1 summarises some of the key aspects of the clinical trials related to the biomarkers and biomarker panels reviewed in this article.

As it can be seen from Figure 2, some of these biomarkers (e.g., S100B) have better sensitivity than others but are less-specific for stroke [32]. In addition, some comorbidities and other factors are also found to interfere with the accuracy of these biomarkers. However, when the biomarkers are combined in a panel, they may offer greater sensitivity and specificity values compared to individual biomarkers [32].

3.2 Biomarker panels

Unavailability of single biomarkers with both high sensitivity and high specificity has been a limiting factor in adopting blood biomarkers as stand-alone diagnostic tools in clinical situations such as stroke. To add to the complexity, patterns of biomarker change may differ depending on the type of stroke (e.g., IS versus ICH) or depending on the affected brain areas [33]. It has been suggested that by combining several biomarkers into a biomarker panel more useful information can be obtained particularly by including biomarkers specific to different areas of the brain [14, 16-18]. In this review, we have identified five biomarker panels that have shown both a sensitivity and specificity of >50% (Figure 3). We have named these five biomarker panels as panels A through E in this

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review due to the lack of specific trade names for them in the original articles (Table 1 and Table 2).

Most of these panels are composed of brain specific biomarkers (neuronal cell activation and neuro-endocrine markers) and non-specific biomarkers (MMP-9, C-reactive protein (CRP), VCAM, vWF, and D-dimer), which represent different components of ischemic cascade and provide complementary information in the diagnosis of stroke. Although the findings from those studies are not conclusive, the use of biomarker panels may have opened a new frontier in the development of highly sensitive and specific biomarkers. Therefore, the concept of diagnostic biomarker panels would be a promising topic for future research.

Table 1. Blood biomarkers for stroke diagnosis

Biomarker	Reference	Sample size (n)	Cut-off	Time from symptoms onset to sample collection (up to)
S100B	Zhou et al., 2016 [19]	46 (ICH) 71 (IS)	67 pg/mL	6hr
GPBB	Park et al., 2018 [17]	172 (S) 133 (C)	7.0ng/mL	4.5hr
Anti-NMDA	Dambinova et al., 2013 [14]	192 (S) 168 (C)	1.0µg/L	3hr
MMP-9	Castellanos et al., 2007 [18]	134 patients	≥140 ng/mL	24hr
APOA1-UP	Zhao et al., 2016 [16]	94 (S) 37 (C)	APOA1-UP/LRP ratio 1.80	72hr
PARK-7	Allard et al., 2005 [15]	622 (S) 165 (C)	9.33µg/L	3hr
NDKA	Allard et al., 2005 [15]	622 (S) 165 (C)	2µg/L	3hr
H-FABP	Park et al., 2013 [13]	111 (S) 127 (C)	9.70 ng/ml	24hr
Panel A	Reynolds et al., 2003 [34]	223 (S) 214 (C)	-	6hr
Panel B	Lynch et al., 2004 [35]	65 (S) 157 (C)	-	6hr
Panel C	Sharma et al., 2014 [21]	167 (S)	-	24hr
Panel D	Laskowitz et al., 2005 [36]	130 (S) 10 (C)	-	6hr
Panel E	Moore et al., 2005 [37]	20 (S) 20 (C)	-	<24 hr (n=7), 24-48 hr (n=10), >48 hr (n=3)

Study (S), Control (C), Intracerebral haemorrhage (ICH), Ischemic stroke (IS), labelled reference peptide (LRP)

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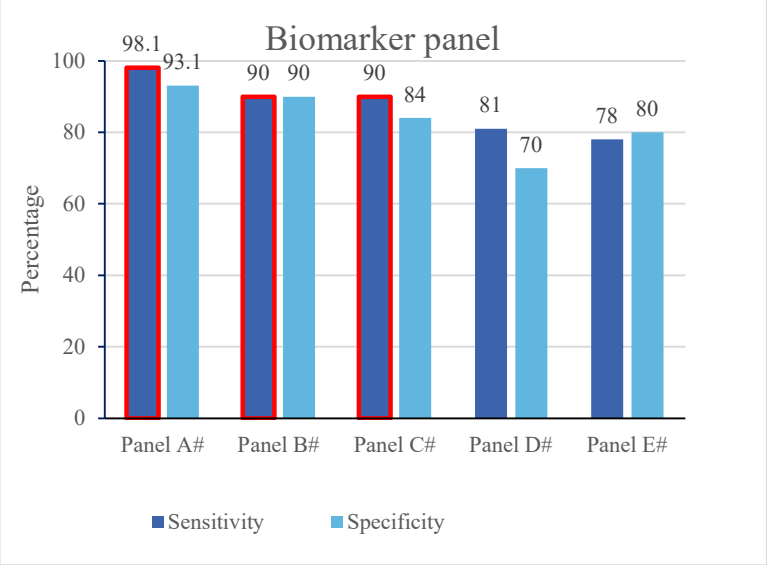

Figure 3. Panel biomarkers for stroke.
Panel A, B and C have sensitivity >90% which is highlighted in red.

Table 2. Panel biomarker composition.

Biomarker Panel	Composition of biomarkers
Panel A (5 proteins)	BNGF, MCP-1, MMP-9, S100B, vWF
Panel B (4 proteins)	S100B, vWF, MMP-9, VCAM
Panel C (5 proteins)	Eotaxin, EGFR, S100A12, Metalloproteinase inhibitor-4, Prolactin
Panel D (5 proteins)	S100B, MMP-9, D-dimer, BNP, CRP
Panel E (22 genes)	Gene names: CD163, Hypothetical protein FLJ22662 Laminin A motif, Amyloid β(A4) precursor-like protein 2, *N*-acetylneuraminate pyruvate lysase, *v-fos* FBJ murine osteosarcoma viral oncogene homolog, Toll-like receptor 2, Ectonucleoside triphosphate diphosphohydrolase 1, Chondroitin sulfate proteoglycan 2 (versican), Interleukin 13 receptor, α1, CD14 antigen, Bone marrow stromal cell antigen 1/CD157, Complement component 1, q subcomponent, receptor 1, Paired immunoglobin-like type 2 receptor α, Fc fragment of IgG, high-affinity Ia, receptor for (CD64), Adrenomedullin, Dual-specificity phosphatase 1, Cytochrome b-245, β polypeptide (chronic granulomatous disease), Leukotriene A4 hydrolase, *v-ets* Erythroblastosis virus E26 oncogene homolog 2 (avian), CD36 antigen (thrombospondin receptor), Baculoviral IAP repeat-containing protein 1 (Neuronal apoptosis inhibitory protein), and KIAA0146 protein

BNGF, B-type neutrotrophic growth factor; MCP-1, monocyte chemoattractant protein-1; MMP-9, matrix metalloproteinase-9; S100B, S100 calcium-binding protein B; vWF, von Willebrand factor; VCAM, vascular cell adhesion molecule; EGFR, epidermal growth factor receptor; S100A12, S100 calcium-binding protein A12; BNP, B-type natriuretic peptide; CRP, C-reactive protein

Panel A is composed of five protein biomarkers that were studied by Reynolds et al (Table 2) [34]. This panel has shown a sensitivity of approximately 98% and specifically

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about 93% for prediction of ischaemic stroke for samples collected within 6 hours from the appearance of symptoms. This is a significant improvement compared to many individual markers in previous studies [34]. Panel B includes four protein biomarkers based on a study by Lynch et al., in 2004. This panel had both 90% sensitivity and specificity where the samples were obtained within 6 hours of the stroke onset [35]. Panel C comprises five protein biomarkers based on a study by Sharma et al., in which they reported a sensitivity of 90%, specificity of 84%, a positive predictive value (PPV) of 78%, and a negative predictive value (NPV) of 93% for stroke detection within 24 hours of symptoms' onset [21]. Panel D, which was developed in a cohort of 130 patients with acute neurological symptoms, consists of five protein biomarkers. This panel showed a sensitivity of 81% and a specificity of 70% for the prediction of IS when the blood specimens were collected within 6 hours of the stroke onset [37]. Given the above information, panels A, B, and D may be clinically useful for triaging purposes [34, 35, 37].

Panel E, made by Moore et al., in 2005 was created following a comparative study of gene expression profiles in confirmed stroke cases (IS; n=20) versus matched healthy controls (n =20) using a microarray technology. Accordingly, and after the initial study of exhaustive gene expression patterns using the RNA samples extracted from peripheral blood mononuclear cells, they observed a significant change (mainly up-regulations) in the expression of 190 genes in patients with IS. Next, a panel of 22 genes was chosen for the derivation of a predictive model for the prediction of stroke using hierarchical cluster analysis. The model was then prospectively validated in another cohort consisting of 9 stroke patients and 10 healthy individuals. This model showed a sensitivity 78% and a specificity of 80% in the validation cohort [37]. These results are promising, however, because of the small samples sizes both for the derivation and the validation studies, the results need to be validated in larger studies.

The above-mentioned biomarkers have not been approved for clinical diagnostic use yet due to several reasons including the lack of large prospective trials, lack of the standards for measurement, unknown interference in certain population groups, or uncertainties in the time-concentration relationships. We believe that the available data are still limited, and explorative investigations such as in vitro studies on stroke biomarkers are still insufficient. We suggest that before starting large-scale clinical studies, we also need to have a better understanding of the window periods of individual biomarkers for stroke detection (the time from symptoms' onset to a detectable change in the blood levels of a biomarker). As we know, the efficacy for current interventions for acute stroke is time-dependent, and most of the current guidelines recommend <6.5 hours as key target between the onset of symptoms and treatment intervention. Therefore, by taking into the consideration the time required for radio-imaging to confirm the diagnosis prior to treatment (which is around 45 minutes in optimal settings and up to 1.5 hours in average settings), any biomarker that can be reliably detected within 5 hours of the onset of stroke could be a highly valuable diagnostic tool.

4. Our study limitations

There are a few limitations for this paper. Firstly, we performed a literature search using studies involving human trials only and excluded animal studies, which may have caused us to miss some of the current literature. Secondly, this was not a systematic review, therefore we may have not identified and reported some other appropriate stroke biomarkers. Thirdly, we only searched for articles published in English, as a result, we might have missed some relevant studies published in non-English languages. Lastly, because most of the patients in the included studies were middle-aged or older adults, some of the conclusions presented here might not be applicable to children and young adults because of age-related differences in the pathophysiology of stroke. Accordingly, we suggest that there is an urgent need for research into the role of blood biomarkers in the detection of stroke in children and young adults.

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5. Conclusions

The results of this literature review indicate that there are potential biomarkers (both as individual biomarkers and as panels) with high-enough sensitivity and specificity that may serve as early detection tools for stroke diagnosis. However, most of the published studies had small sample sizes, which makes the clinical applicability of their findings challenging. Therefore, further research needs to be done in larger cohorts to confirm the clinical usefulness of the available data. In addition, most of the proposed biomarkers have not been examined in very acute patients within the first 3 - 5 hours post-stroke, hence the need for further research in this area.

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