MicroRNAs as Biomarkers in Spontaneous Intracerebral Hemorrhage: A Systematic Review of Recent Clinical Evidence

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Abstract

Objective:

Spontaneous intracerebral hemorrhage (SICH) is a growing subtype of stroke associated with high mortality and devastating disabilities. Therefore, identifying non-invasive biomarkers for SICH would have a tremendous clinical impact. MicroRNAs (miRNAs) are non-coding single-stranded RNAs containing 21 to 23 nucleotides and control various protein-coding genes' activity through posttranscriptional repression. In this systematic review, we aimed to report the recent clinical evidence on the role of microRNAs as biomarkers for prediction, prognosis, early detection, and risk stratification of SICH.

Methods:

We conducted a systematic search on PubMed and Embase databases and included only full-text peer-reviewed articles published in English.

Results:

We included 10 studies: Seven case–control studies, two cohort studies, and one cross-sectional study. There were 27 altered miRNAs, suggesting their role as biomarkers for the early detection of ICH. Additionally, 34 miRNA expressions were associated with poor prognosis of ICH; miR-126 and miR-23a-3p expressions correlated with relative perihematomal edema (PHE) volume and, using a subset of 10 miRNA signatures, had an accuracy of 100% in predicting hematoma in patients with ICH. MiR-4317 and miR-4325 profiling were predictable of the development of late seizures. There were 39 miRNAs associated with the incidence of all types of strokes, while 10 miRNAs correlated with the predicted risk of stroke but were not specific to a stroke subtype. The altered miRNA signatures contributed to endothelial dysfunction, hematoma, and PHE

through leukocyte activation, oxidative stress response, programmed cell death, smooth muscle cell proliferation, and apoptosis of cerebrovascular endothelial cells.

Limitations to this review included; the paucity of studies and the lack of randomized trials; also, there could be selection bias in the prospective studies included. Additionally, correlations are possible between the outcomes and other factors such as therapeutic interventions and ICH severity; also between the circulating miRNA profiles and gene expression profiles, and other undergoing pathological conditions. There are also some limitations regarding the methods of obtaining miRNAs and identifying target RNAs specific to SICH pathology. Finally, the prediction and risk of stratification SICH couldn't be calculated separately from IS.

Conclusion:

There were alterations in various miRNA signatures, suggesting their potential role as biomarkers for early detection and differentiation of SICH; miRNA expressions were also associated with poor prognosis of SICH. In addition, miRNAs correlated with the predicted risk of stroke but were not specific to a stroke subtype. Further studies are needed, especially on the therapeutic potentials of miRNAs and their target RNAs in SICH.

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1. INTRODUCTION

Spontaneous intracerebral hemorrhage (SICH) accounts for 10–15% of strokes. It is associated with a mortality rate of up to 50% in the first 30 days and devastating permanent neurological disabilities in 75–80% of survivors [1-3]. The incidence of SICH has grown to 24.6 per 100,000 person-years and continues to escalate due to the increased use of anticoagulation and antiplatelet drugs and increasing population age [4,5].

While neuroimaging modalities, particularly computed tomography (CT), are the standard diagnostic methods for diagnosing and localizing strokes, such imaging is not available in all emergency centers and is not commonly available in prehospital settings. Moreover, the means for predicting hemorrhagic strokes (HSs) in high-risk individuals are lacking [6,7]. Therefore, identifying non-invasive biomarkers for SICH would be clinically meaningful.

Aside from environmental and vascular risk factors, genetic factors play a role in the risk of stroke development [8,9]. Among genetic factors, such as the 12q24.2 locus and *ABO*, which account for a proportion of the heritable risk, evidence increasingly indicates that microRNAs (miRNAs) play a critical role in the pathophysiology of stroke [10-13].

miRNAs are evolutionarily conserved non-coding single-stranded RNAs containing 21–23 nucleotides and are present in many human cell types and extracellular spaces, including blood [14,15]. Accumulating evidence suggests that miRNAs control the activity of more than 60% of all protein-coding genes by repressing protein synthesis post-transcriptionally [16]. They pair with the messenger RNAs (mRNAs) of protein-coding genes and destabilize them by poly(A)-tail shortening (deadenylation), mRNA decay, or interfering with mRNA-ribosome interactions causing translational repression, as illustrated in Figure 1 [17,18]. Furthermore, deregulation of circulating miRNAs is a risk factor for atherosclerosis and coronary heart

diseases [19,20]. This disrupts their functions as modulators and fine-tuners of various pathophysiological mechanisms and signaling pathways [20]. Moreover, miRNA signature profiling showed that some signatures correlated with brain tumor diagnosis, prognosis, and treatment response in humans [21].

This systematic review aims to report recent clinical evidence of circulating miRNAs as biomarkers for prediction, risk stratification, early detection, and prognosis of SICH.

2. MATERIALS AND METHODS

2.1 Search Strategy

This systematic review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. This review was registered in the International Prospective Register of Systematic Reviews (PROSPERO) with the registration number (CRD42021261950) [22]. As shown in Figure 2, we conducted a systematic search on PubMed and Embase databases on June 21, 2021. We searched for randomized controlled trials and observational studies using Medical Subject Headings (MeSH) terms and keywords for the topic of interest, such as “microRNAs,” “intracranial hemorrhage,” and “HS,” separately and in combination, as shown in (Appendix 1). Only full-text peer-reviewed randomized controlled trials, case-control, cohort, and cross-sectional studies that were published from June 21, 2011, to June 21, 2021, in English, which investigated the role of circulating miRNAs in the prediction, early detection, risk stratification, and prognosis of SICH in adult humans were considered eligible. The search strategy was adapted to the appropriate syntax of each database platform. We excluded articles in languages other than English, animal studies, reviews, gray literature, case reports, case series, letters to the editor, books, documents, and studies published before June 2011.

2.2 Data Extraction and Data Analysis

Two reviewers (WS and LGDDM) independently screened the retrieved records by title, abstract, and full text to identify articles for eligibility, with FB as the arbiter. The selected

articles were analyzed using a standardized data extraction form and were assessed using the Newcastle-Ottawa bias scale tool for quality assessment [23]; studies with total scores of less than six were excluded.

3. RESULTS

Our initial search on PubMed and Embase databases resulted in 25,829 articles published in English over the last 10 years. Duplicates were identified and eliminated, and automatic filters were applied. Articles were screened by title and abstract, and potentially relevant articles were evaluated for eligibility by screening the full text and by applying quality assessment tools. As a result, 25,819 articles were excluded, and the remaining eligible 10 articles that investigated the role of mRNAs in ICH were included in this systematic review. Table 1 shows the included studies and the Newcastle-Ottawa risk of bias scores achieved. We included seven case-control, two cohort studies, and one cross-sectional study, as summarized in Table 2.

3.1 Alterations in miRNA Profiles in Patients with Intracerebral Hemorrhage (ICH)

The seven case-control studies compared circulating miRNA profiles, measured by a quantitative real-time polymerase chain reaction (qPCR) in plasma (in six studies) and whole blood (in one study), of 305 patients with ICH [7,24-29]. ICH was confirmed by CT or magnetic resonance imaging (MRI) with 165 matched controls. Target mRNAs were identified, and functional analysis was performed. They identified 152 miRNAs with altered expression (62 upregulated; 93 downregulated) that could potentially serve as biomarkers for ICH. Moreover, in the two cohort studies, which included 1,959 individuals identified 47 miRNAs with altered expressions were identified in patients with ICH as compared to controls [30], and 39 miRNAs that were associated with the incidence of stroke (all types) over a mean follow-up of 9.7 years ($p < 0.05$), were identified [31]. In a cross-sectional study that included 1,523 participants, 10 miRNAs with altered expression were identified [32]. Of these, three correlated positively, and seven correlated negatively with the predicted risk of stroke ($p < 0.05$), but these were not specific for a stroke subtype [32].

3.2 Role of miRNA in the Early Detection and Differentiation of ICH

Two case-control studies compared miRNA profiling in 34 patients with ICH with 90 patients with ischemic stroke (IS) and 31 healthy controls and identified six differentially expressed miRNAs that could help in early detection and differentiation of ICH from IS [7,24]. In patients with HS, the median plasma concentration of miR-124-3p was 94% higher than in patients with IS and 155% higher than in controls [7]. In patients with IS, the plasma concentration of miR-16 was 24% higher than in patients with HS and 35% higher than in controls [7]. MiR-124-3p levels were higher in patients with moderate to severe HS, while miR-

16 levels were higher in patients with mild stroke [7]. Additionally, concentrations of miR-124-3p correlated positively with lesion volume on CT (r = 0809, $p = 0.0005$) in patients with HS [7]. Similarly, among the 13 altered miRNAs detected by Guo et al. [24], the levels of miR-27a, miR-365, miR-150, and miR-34c-3p were significantly increased in the plasma of patients with ICH compared to those in patients with IS and the controls ($p < 0.05$).

Other potential miRNAs for the early detection of ICH were identified in a case-control study that compared miRNA profiles in 106 patients with spontaneous non-aneurysmatic ICH with 50 healthy controls [25]. They identified three miRNAs that were upregulated and one that was downregulated in patients with ICH. The diagnostic sensitivity and specificity of miR-145 and miR-181b for the detection of ICH, and the areas under the curve were 0.766 (95% CI, 0.689–0.838) and 0.78 (95% CI, 0.70–0.86), respectively, suggesting that circulating miR-145 and miR-181b are potential biomarkers for the diagnosis of ICH.

Additionally, a case-control study included 23 patients with ICH and 17 matched vascular risk factor controls [26] and identified 29 miRNAs (22 down, seven up), 250 target mRNAs (136 up, 114 down), and seven small nucleolar RNAs that were altered in patients with ICH compared to controls (15 were downregulated, two were upregulated at ≤ 72 h) that can help in the early detection of ICH.

3.3 miRNAs and Prognosis of ICH

Three case-control studies identified 32 miRNAs, of which expression levels were associated with a poor prognosis of ICH [27-29]. In 27 patients with ICH, miR-126 expression levels were positively correlated with the relative perihematomal edema (PHE) volume on days 3–4 ($r = -0.714$; $p < 0.001$), as compared to 11 controls [27]. In another study, in 30 hospitalized

patients with ICH, upregulation of miR-23a-3p on the 3rd day was significantly correlated with relative PHE volumes in patients with ICH ($p = 0.0002$), predisposing them to worse outcomes [28]. In 79 patients with ICH, 30 miRNAs were altered in patients with or without secondary hematoma ($p < 0.05$); 19 of these were upregulated, and 11 were downregulated in the hematoma group [29]. The signature of a subset of five of these miRNAs attained an accuracy of 90%, while that of a subset of 10 miRNAs reached an accuracy of 100% in predicting hematoma in patients with ICH [29]. Additionally, in a cohort study that included 15 patients with ICH and three healthy controls, over the follow-up period of 14 days to one year, miR-4317 and miR-4325 were consistently and significantly downregulated in the whole blood in those with post-ICH late seizures vs. those without seizures at one year ($p < 0.05$). Thus, these miRNAs can serve as biomarkers for the prognosis of ICH [30].

3.4 Role of miRNAs in the Prediction and Risk Stratification of ICH

In a cohort study that included 1,914 individuals with no history of stroke [31], over a 9.8-year follow-up period, 138 individuals were diagnosed with stroke, including 19 patients with HS and 96 patients with IS. Based on neuroimaging, 39 miRNAs were associated with the incidence of stroke. There were significant associations between the expression levels of three miRNAs (miR-6124, miR-5196-5p, and miR-4292) and an increased risk of HS in the general population [31]. The limitations of this study included the fact that the miRNAs were not specific to HS, that the number of cases of HS was small, and that the 95% CI was wide. A cross-sectional study that included 1,523 participants identified 10 miRNAs, of which three correlated positively and seven correlated negatively with the predicted risk of stroke ($p < 0.05$) [32]. In 45 controls and 86 cases of cerebrovascular disease (CVD), seven miRNAs were significantly

associated with CVD ($p < 0.05$). A set of three miRNAs (miR-1268b, miR-4433b-3p, and miR-6803-5p) were the best predictor models for stroke risk, with 80% sensitivity and 82% specificity. However, these could not be used to calculate the separate risks of HS and IS.

4. DISCUSSION

Our review showed that multiple miRNA signatures in the blood were altered in patients with ICH as compared to controls [7,24-32]. Additionally, miRNAs were associated with the inflammatory response, arterial stiffening, cerebrovascular endothelial dysfunction, hematoma, PHE formation, and development of seizures and were suggested to predict CVD before the onset of stroke [25-32]. This indicates the potential role of miRNAs as biomarkers for the early detection, prognosis, prediction, and risk stratification of ICH.

The recent literature suggests that miRNAs can be used as biomarkers for multiple diseases, including brain tumors [21], and minimally invasive lung cancer [33]; moreover, some studies have reported miRNAs to be related to the pathology of atherosclerosis and coronary heart disease [19] and to the pathogenesis of IS [34]. Therefore, miRNAs and their target mRNAs can be further used as therapeutic agents. For example, Ferreira et al. [35] showed the potential use of miR-18a to improve endothelial cell functions and treat cerebral arteriovenous malformations. Additionally, miRNAs are targets for cancer therapy through mechanisms that include the inhibition of miRNAs that are upregulated and the replacement of miRNAs that are downregulated in cancers [36].

Arterial stiffening and endothelial dysfunction play significant roles in CVDs [37]. Arterial stiffening results from many diseases, particularly hypertension and atherosclerosis [38]. Arterial stiffening plays a crucial role in the pathogenesis, incidence of complications, and prognosis of ICH [39]. Gareev et al. identified one upregulated (miR-145) and one downregulated (miR-181b) miRNA that can not only be used as biomarkers for spontaneous ICH, but that also play important roles in the pathogenesis of ICH [25]. miR-145 is a modulator of the smooth muscle phenotype in response to injury. Changes in its expression will alter the

progression of atherosclerosis by affecting the formation of the neointima. Also, it is overexpressed in atherosclerotic plaques in patients with hypertension [40,41]; this may be a compensatory response to chronic high blood pressure and atherosclerosis of the cerebral vessels [25]. miR-181b plays multiple neuroprotective roles in ICH; it targets importin-α3, regulates the downstream nuclear factor-κB (NF-κB) signaling in the vascular endothelium [42], and inhibits Notch1 expression in the Notch signaling pathway, protecting endothelial cell function [43]. Additionally, in vitro, it attenuates apoptosis of neurons and caspase-3 activity and neuronal necrosis and apoptosis induced by erythrocyte lysates; in vivo, the downregulation of miR-181b increases proinflammatory cytokines, brain edema, and neurological injury after ICH by increasing the level of heat shock protein A5 in ICH [44]. ICH evokes local and systemic inflammation, in which blood cells form leukocyte-platelet aggregates, adhere to brain endothelial cells, and infiltrate the brain, resulting in blood-brain barrier disruption, brain edema, and parenchymal brain injury, which worsens the outcomes [26].

Cheng et al. identified the functional analyses of five downregulated miRNAs (miR-146a-5p, miR-210-5p, miR-93-5p, miR-17-3p, and miR-378a-5p) and their target mRNAs [26]. They showed that the targeted upregulated mRNAs were associated with various molecular signaling pathways, including toll-like receptor, natural killer cells, focal adhesion, tumor growth factor-β, adipocytokine, fatty acid metabolism, cytokine–cytokine receptor, chemokine, apoptosis, vascular smooth muscle, phagosome, Janus kinase signal transducer and activator of transcription proteins (JAK-STAT), and RNA degradation pathways.

MiRNAs play a critical role in the pathogenesis and, subsequently, prognosis and complications of ICH, such as hematoma and PHE, which occur early in the acute and subacute stages of ICH and which are strongly associated with poor outcomes. In particular, PHE may be

more involved in poor neurological outcomes [45]. PHE can significantly enlarge within 7–11 days and can last for long periods, further increasing the hematoma space-occupying effect, leading to intracranial hypertension and hernia, which are associated with poor prognosis [46,47]. Four downregulated miRNAs that have been associated with ICH-target genes (miR-126, miR-146a, miR-let-7a, and miR-26a) were identified by bioinformatic data mining, and gene functional annotation analysis showed that these genes are connected to biological processes involved in the innate immune response, leukocyte activation (miR-126 and miR-146a), response to oxidative stress (miR-146a), programmed cell death (miR-let-7a), and smooth muscle cell proliferation (miR-26a) [27].

Furthermore, miRNA-23a-3p plays a role in the development of cerebral edema by disrupting tight junctions and regulating the proliferation and apoptosis of cerebrovascular endothelial cells [28,48,49]. This can be explained by the reduction in the mRNA levels of zonula occludens-1 (*ZO1*) in cerebrovascular endothelial cells after the miRNA-23a-3p knockdown. *ZO1* silencing RNA inhibits proliferation and promotes the apoptosis of cerebrovascular endothelial cells. This suggests that upregulation of miRNA-23a-3p worsens the prognosis of ICH, as it increases brain edema by increasing *ZO1* expression [28]. Controlling PHE and hematoma formation can be important adjunctive therapeutic targets to improve the outcome of patients with ICH.

Patients with ICH are at high risk for the late development of seizures, even when compared with patients with IS; 15% of patients with ICH experience late seizures [50,51]. MiR-4317 and miR-4325 were found to be downregulated in post-ICH late seizures vs. non-seizure. Promoting innate inflammatory pathways is associated with late seizures after ICH [30]; miR-4325 downregulation correlates with increased expression of the mitogen-activated protein

kinase-8 (MAPK8) pathway [52], which activates tumor necrosis factor-α (TNF-α), interleukin-1β, interleukin-6, and cyclooxygenase-2. Further, decreased expression of COMM-domain containing 6 (COMMD6), which inhibits the activation of NF-κB by TNF-α, was observed in post-ICH seizures [30,53]. Moreover, miR-4317 targets SL38A1, which is a critical glutamine transporter required for glutamate synthesis, and downregulation of miR-4317 leads to an increase in the synthesis of glutamate [30]. Excess glutamatergic neurotransmission is involved in epileptogenesis and refractory epilepsy [54,55]. Extracellular glutamate provokes neurotoxicity, causing excessive calcium entry into the cell through overstimulation of glutamate receptors, which in turn leads to excitotoxicity and neuronal death [56,57]. Downregulated miR-4325 and miR-4317 have been suggested to be prognostic biomarkers for the prediction of late post-ICH seizures, and the biological pathways in which they are involved are potential therapeutic targets for seizures.

Table 3 summarizes the altered miRNA signatures, their functions, target genes, and potential role as ICH biomarkers.

4.1 Limitations

To the best of our knowledge, there has been no previous systematic review reporting on the clinical evidence of miRNAs as biomarkers for ICH. However, this study has some limitations. First, there were a limited number of high-quality studies in humans, and no randomized trials were found; moreover, the prospective studies that were included may have had some selection bias. Additionally, some studies included a small number of patients; thus, larger sample sizes are needed in future studies, and results should be cautiously interpreted until then. In addition, correlations are possible between the outcomes and other factors such as therapeutic

interventions and ICH severity, also the association between the circulating miRNA profiles and gene expression profiles, and other undergoing pathological conditions. There are also some limitations in regards to obtaining tissue-specific miRNA levels via quantitative polymerase chain reaction methods and identifying target RNAs specific to SICH pathology using target databases. Finally, studies on the role of miRNAs in the prediction and risk stratification of ICH did not calculate the separate risk of HS.

5. CONCLUSIONS

There is a crucial need for more diagnostic and prognostic biomarkers for ICH. This review describes the various roles of miRNA profiles for identifying potential biomarkers of ICH. Based on the literature to date, 27 miRNA signatures show alterations in ICH, suggesting their role as biomarkers for the early detection and differentiation of ICH. Additionally, the expression of 34 miRNAs correlated with poor prognosis of ICH, including hematoma and PHE formation, and the late development of seizures (within the following year). Moreover, among the 39 miRNAs that were associated with the incidence of stroke, there were significant associations between the levels of three miRNAs and increased risk of HS in the general population, while 10 miRNAs were correlated with the predicted risk of stroke but were not specific to HS. Circulating miRNA profiles in patients with ICH showed alterations that were associated with arterial stiffening, endothelial dysfunction, hematoma, and PHE formation, by modulating the innate inflammatory response, suggesting that miRNAs also play a role in the pathophysiology of ICH. Plasma-derived miRNAs were more favorable to blood-derived miRNAs as they were more specific to the SICH pathology.

Overall, there is limited knowledge on the pathogenesis of ICH as compared to IS. Therefore, it is imperative to explore the alterations in miRNA signatures and their role in ICH.

Additionally, further investigations are needed to focus on the therapeutic potential of miRNAs and their target RNAs for preventing ICH and on the development of management protocols that aim to prevent secondary brain damage and other unfavorable outcomes in ICH.

Authors' contributions

Waleed Sultan: Conceptualizing, Methodology, Visualization, Writing–Review and Editing. **Luiz Gabriel Dias Duarte Machado:** Conceptualizing, Methodology, Writing–Review and Editing. **Mohamed G Ali:** Investigation, Validation, Writing–Reviewing & Editing. **Alessio Tramontana:** Conceptualizing, Methodology, Writing–Reviewing & Editing. **Ahmed Ezzet Bayoumy:** Validation, Reviewing and Editing. **Silvia Gesheva Baxter:** Methodology, Reviewing and Editing. **Mahmoud Ramadan Adly Aly:** Visualization, Reviewing and Editing. **Federico Bilotta:** Supervision, Conceptualizing, Methodology, Reviewing and Editing.

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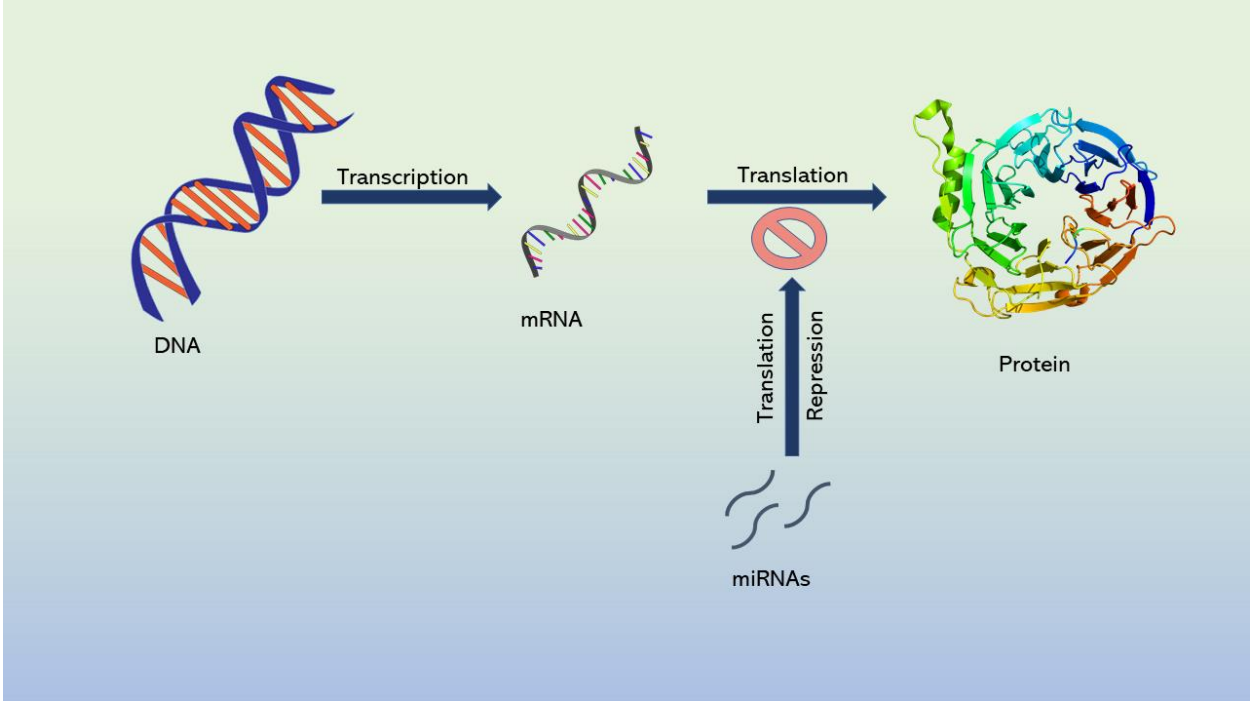

Figure 1. microRNAs (miRNAs) inhibit the translation of the target messenger RNA (mRNA) and repress protein synthesis.

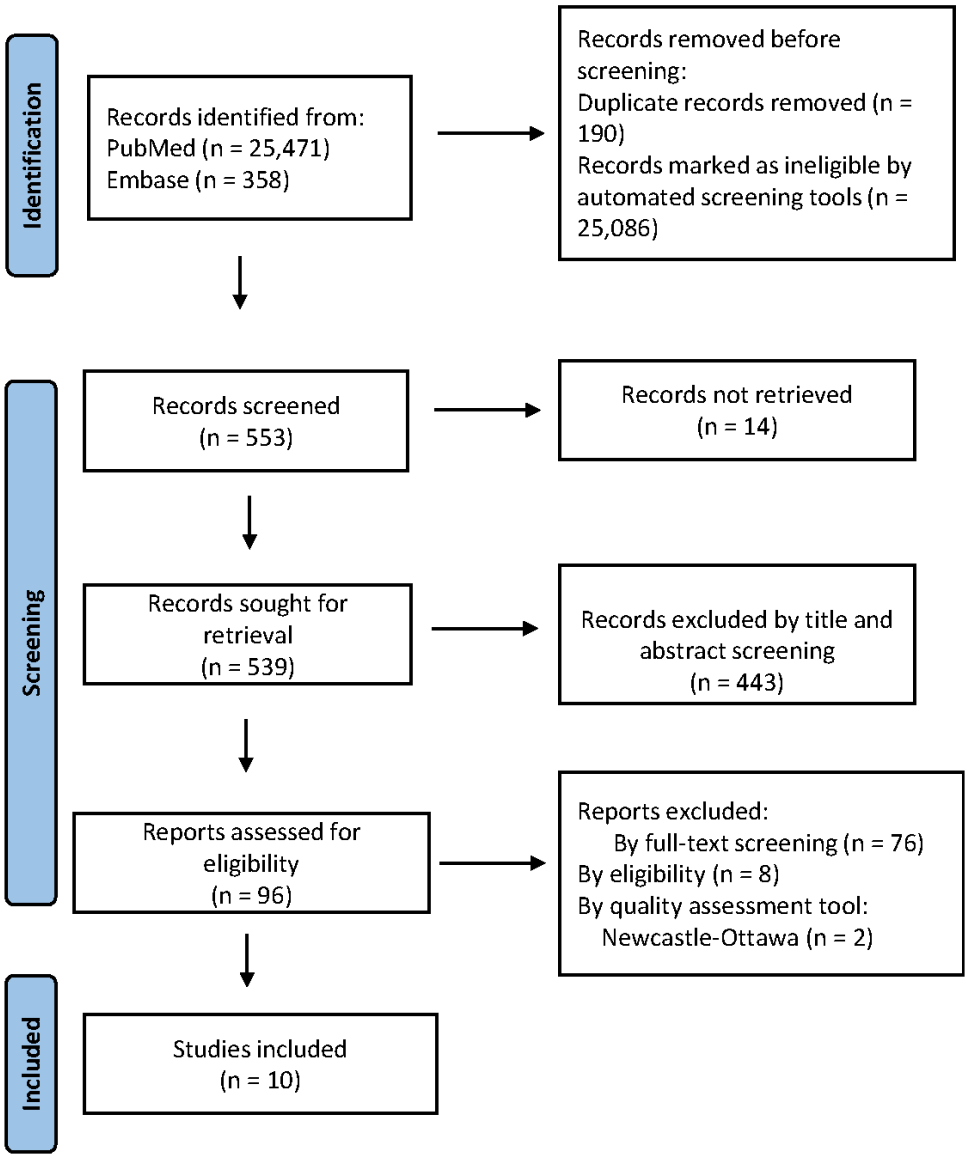

Figure 2. PRISMA flow diagram (2020) of the literature search.

PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Author and year	Ref.	Study type	Bias risk tool	Scoring			Final score (9) and bias risk assessment
				Selection (4)	Comparability (2)	Exposure/ outcome (3)	
Leung et al., 2014	7	Case-control	Newcastle-Ottawa	4	2	2	8/low bias risk
Guo et al., 2013	24	Case-control	Newcastle-Ottawa	3	2	2	7/low bias risk
Gareev et al., 2020	25	Case-control	Newcastle-Ottawa	4	1	3	8/low bias risk
Cheng et al., 2019	26	Case-control	Newcastle-Ottawa	3	1	3	7/low bias risk
Zhu et al., 2015	27	Case-control	Newcastle-Ottawa	3	2	2	7/low bias risk
Hu et al., 2018	28	Case-control	Newcastle-Ottawa	3	2	2	7/low bias risk
Zheng et al., 2012	29	Case-control	Newcastle-Ottawa	4	2	2	8/low bias risk
Iwuchukwu et al., 2019	30	Cohort	Newcastle-Ottawa	3	2	2	7/low bias risk

Mens et al., 2021	31	Cohort	Newcastle-Ottawa	3	2	2	7/low bias risk
Sonoda et al., 2019	32	Case-control within cross-sectional	Newcastle-Ottawa	3	2	2	7/low bias risk

Table 1. The included studies, risk of bias tools, and assessment scores.

Author	Ref	Type	Purpose	Study characteristics	Outcomes
Leung et al. 2014	7	Case-control	MiRNAs as biomarkers for early detection of ICH and differentiation from IS.	HS (n = 19) or IS (n = 74) confirmed by CT or MRI, and healthy controls matched by sex and age (n = 23). The median age was 72 years; 50.5% were males; all presented, and samples were collected within 24 h of symptoms. Logistic regression analysis was performed	– miR-124-3p in the plasma was 94% higher in the HS group than in the IS group (p = 0.0109) and 155% higher than controls (p = 0.0194) – miR-16 was 24% higher in the IS group than in the HS group (p = 0.0399) and 35% higher than controls (p = 0.0088). – miR-124-3p was higher in moderate to severe stroke (p = 0.0013) – miR-16 was higher in mild stroke

					(p = 0.0088) – miR-124-3p correlated positively with lesion volume on CT (r = 0809, p = 0.0005) in HS
Guo et al. 2013	24	Case-control	MiRNA signature changes in the plasma of patients with ICH.	Patients with ICH (n = 15), patients with IS (n = 16) confirmed by CT or MRI, and eight healthy volunteer controls. Blood samples were collected 1–14 days from onset of symptoms (median 6 days). A separate cohort of 12 controls (n = 12) and ICH patients (n = 11) were used for the validation	– miR-365, miR-27a, miR-150, and miR-34c-3p were higher in patients with ICH than patients with IS and controls (p < 0.05) – In controls, miRNAs were only in the microvesicle fraction, whereas in ICH, the upregulated miRNAs were in the microvesicle and the supernatant fractions of the plasma.

				experiments. Linear regression analysis was performed.	
Gareev et al., 2020	25	Case-control	MiRNAs as diagnostic biomarkers for the detection of ICH.	Spontaneous non-aneurysmatic ICH patients (n = 106) and healthy volunteer controls (n = 50) without a history of cancer and cerebrovascular or cardiovascular disease as controls. Blood samples were collected within 24 hours of admission. Chi-square test , t-test, analysis of variance, or the Mann-Whitney U were used when	– miR-181b was lower in patients with ICH ($p <$ 0.001) Diagnostic sensitivity and specificity (95% CI, [0.70–0.86]) – miR-223 ($p <$ 0.05), miR-155 ($p <$ 0.05), and miR-145 ($p< 0.001$) were higher in patients with ICH – Circulating miR-145 (AUC 0.766; 95% CI, 0.689–0.838) and miR-181b (AUC 0.78;

				appropriate.	95% CI, 0.70–0.86) showed marked accuracy as biomarkers for ICH diagnosis.
Cheng et al., 2019	26	Case-control	The detection of changes in miRNAs and their target mRNA levels in ICH patients' whole blood.	Patients with ICH (n = 23) and VRFC (n = 17) matched for age, sex, race, diabetes, hypertension, hyperlipidemia, and atrial fibrillation. Whole blood samples from three groups (ICH at ≤ 72 h, n = 14; ICH at >72 h, n = 9; and VRFC, n = 17). A univariate analysis was performed.	– 29 miRNAs (22 down, seven up), 250 target mRNAs (136 up, 114 down), and seven small nucleolar RNA were altered in patients with ICH compared to controls. – Fifteen were downregulated (miR-146a-5p, miR-210-5p, miR-93-5p, miR-17-3p, miR-378a-5p, miR-221, let-7i, miR-378c, miR-378d, miR-378e, miR-378i,

					miR-532-5p, miR-874, miR-4450, and mir-4707), and two were upregulated (Mir-1183 and let-7d-3p) at < 72 h ($p < 0.05$) – Five miRNAs (miR-146a-5p, miR-210-5p, miR-93-5p, miR-17-3p, and miR-378a-5p) showed an association with mRNA networks involved in inflammatory response in ICH.
Zhu et al., 2015	27	Case-control	MiRNA profiles associated with perihematomal	Patients with ICH (n = 33, 13 males and 20 females; median age, 57.37 ± 9.67 years) and control	– 55 miRNAs (54 down, one up) were altered in patients with ICH ($p < 0.05$). – MiR-126, MiR-

			edema formation in patients with ICH.	(n = 15) age- and sex-matched healthy volunteers. Two cranial CT scans were performed on all patients at (< 24 h; initial) and 3–4 days after the onset of symptoms (follow-up). Blood samples were collected from patients on admission and from controls upon entering the study. Chi-square test or Fisher's exact test for categorical Variables; student's t-test, or Mann–Whitney U test for continuous	146a, MiR-let-7a, and MiR-26a were significantly lower in 27 patients with ICH than 11 controls ($p < 0.01$). – MiR-126 correlated with relative PHE volume on days 3–4 ($r = -0.714$; $p < 0.001$) in patients with ICH.

				variables, and Spearman's correlation analysis were performed.	
Hu et al., 2018	28	Case-control	The prediction of perihematomal edema formation after ICH.	Adult patients with ICH (n = 30) admitted within 24 h without infratentorial intracerebral hemorrhage, intraventricular hemorrhage, hematoma enlargement, secondary cerebral hemorrhage, hematoma aspiration after intracerebral hemorrhage, and recent cerebral infarction within the	– miR-23a-3p and MiR-130a were upregulated, whereas MiR-26a and MiR-146a were downregulated in patients with ICH compared to controls. – Upregulation of miRNA-23a-3p on the third day was correlated with relative PHE formation after ICH (p = 0.0002)

				previous 3 months, and control (n = 30) patients matched for age, sex, and basic diseases were included. Blood samples were collected 3 days after admission. Chi-square and *t*-test were used.	
Zheng et al., 2012	29	Case-control	MiRNAs as biomarkers to predict secondary hematoma enlargement in patients with ICH.	Seventy-nine patients with ICH and normal controls in the following groups: hematoma volume growth >33% or > 12.5 mL group (n = 30), matched cases without hematoma growth (n = 49), and	– Thirty miRNAs were altered among patients with or without HE ($p < 0.05$); 19 were upregulated, and 11 were downregulated in the HE. – A subset of five miRNA signatures showed 90%

				healthy control group (n = 30). Blood samples were collected on admission. Multivariate regression analysis was used.	accuracy, whereas a subset of 10 miRNA signatures showed 100% accuracy in predicting hematoma.
Iwuchukwu et al., 2019	30	Cohort	MiRNAs as biomarkers for prediction of late seizures after ICH.	Fifteen patients with ICH with a history of hypertension, late seizure (n = 6), and no-seizure (n = 9). Mean age 59.5 years (SD 12.2) 60% males and healthy controls (n = 3) The follow-up period was 14 days to 1 year. Interviews were conducted via	– miR-4325, miR-181a-5p, miR-1180-3p, and miR-4317 were identified as potential predictors of post-ICH late seizure. – miR-4317 and miR-4325 were downregulated in post-ICH late seizures vs. non-seizure at 1 year ($p < 0.05$).

				phone calls, and electronic medical records were reviewed. Blood samples were collected within 48 h of presentation. Benjamin–Hochberg method of multiple testing corrections was used to arrange data by statistical significance.	
Mens et al., 2021	31	Cohort	MiRNAs as biomarkers for risk prediction of stroke.	The study included 1,914 individuals with no history of stroke (mean age 71.5 years ± 7.6; 57.7% women) within the Rotterdam	– Identified 39 miRNAs that were associated with stroke ($p < 0.05$). – Significant associations between the expression of

				prospective population-based Study. Participants were followed up for a mean of 9.7 ± 3.2 years for the incidence of stroke;138 individuals were diagnosed with stroke: ischemic stroke (n = 96), HS (n = 19), based on neuroimaging; unspecified strokes lacked neuroimaging (n = 23). Cox proportional hazards regression was used to adjust for confounders.	miR-6124 (HR, 2.5 [95% CI, 1.7–3.68], $p = 3.61 \times 10^{-6}$), miR-5196-5p (HR, 3.13 [95% CI, 1.88–5.21], $p = 1.15 \times 10^{-5}$), miR-4292 (HR, 4.87 [95% CI, 1.66–14.33], $p = 4.3 \times 10^{-3}$), and HS.

Sonoda et al., 2019	32	Cross-sectional and case–control	MiRNAs as biomarkers for risk prediction of stroke.	Individuals ≥ 40 years (n = 1612) who underwent a medical checkup with no history of stroke or cancer and no hospitalization during the preceding 3 months in 2015. Group 1 (n = 1523): aged 40–69 years with no history of stroke, randomly divided into Group 1A and Group 1B with matched risk factors. Group 2 (n = 89) aged > 69 years with no history of stroke. Group 3 (n = 173):	– Three (miR-1228-5p, miR-1343-5p, and miR-6805-5p) positively correlated and seven (miR-1268a, miR-1268b, miR-4433b-3p, miR-6089, miR-6090, miR-6752-5p, and miR-6803-5p) negatively correlated with the predicted risk of stroke ($p < 0.05$). – Seven of the 10 miRNAs (miR-1228-5p, miR-1268a, miR-1268b, miR-4433b-3p, miR-6090, miR-6752-5p, and miR-6803-5p) were significantly

				>69 years with CVD; Groups 2 and 3 were further divided into A and B subgroups. Fisher linear discriminant analysis was used to generate diagnostic indices.	associated with CVD ($p < 0.05$). – The model of three miRNAs had 80% sensitivity and 82% specificity (AUC 0.88; 95% CI, 0.83–0.95) for the prediction of CVD.

Table 2. Summary of the type, purpose, characteristics, and outcomes of the included studies. miR, microRNA; IS, ischemic stroke; HS, hemorrhagic stroke; ICH, intracerebral hemorrhage; CVD, cerebrovascular disease; CI, confidence interval; AUC, area under the curve.

Biomarker	Effect	Potential use	Reference
mir-16 **miR-124-3p**	Induces apoptosis via targeting Bcl2. Brain-specific, released from damaged brain tissue.	Detection and differentiation from IS	7
miR-365, **miR-27a,** **miR-150,** **miR-34c-3p**	Inflammatory response modulation.	Detection	24
miR-181b **miR-145**	Decreases leukocyte infiltration in the vascular endothelium, decreases vascular inflammation via targeting NF-kB. The development of vascular stiffness via targeting TGF-β, pSMAD2/3. Modulates the VSMC phenotype and proliferation in response to vascular injury via targeting SRF. Modulates the VSMC phenotype and proliferation in response to	Detection Detection	25

	vascular injury via targeting CD40. Decreases vascular inflammation via targeting NF-kB. Increases neuronal cell death via targeting Nurr1 and TNF-a.		
miR-146a-5p **miR-210-5p** **miR-93-5** **miR-17-3p** **miR-378a-5p**	Various molecular signaling, including Toll-like receptor, natural killer cells, focal adhesion, tumor growth factor-β, adipocytokine, fatty acid metabolism, cytokine–cytokine receptor, chemokine, apoptosis, vascular smooth muscle, phagosome, JAK-STAT, and RNA degradation.	Detection	26
miR-126 **miR-146a** **miR-let-7a** **miR-26a**	Leukocyte activation. Response to oxidative stress and leukocyte activation. Programmed cell death. Smooth muscle cell proliferation.	Detection, prognostic for PHE formation Detection	27

miR-23a-3p	Regulating proliferation and apoptosis of cerebrovascular endothelial cells via *ZO1*.	Prognostic for PHE formation	28
miR-1249, miR-574-5p, miR-1290, miR-522, miR-130a, miR-1202, has-let-7f-2, miR-586, miR-122, miR-29c	Apoptosis, cell proliferation regulation, immune, hypoxia response, collagen biosynthesis, vascular development regulation, water homeostasis and electric ion channel activity regulation, energy metabolic process and coagulation, extracellular matrix constituent, cell surface binding, blood pressure control, and inflammation activation.	Prognostic for hematoma formation	29
miR-4325 **miR-4317**	Increases the expression of the MAPK8 pathway. MAPK8 activates TNF-α, IL-1β, IL-6, and cyclooxygenase-2. Increases the synthesis of glutamate via targeting SL38A1.	Prognostic for late seizures	30
miR-6124	Targets carious genes; CASZ1 (involved in blood pressure), COL15A1, HDAC9 (involved in	Risk stratification and prediction	31

	coronary artery disease), NRP2, RERE, SH3PXD2A, SLC4A8, STXBP5, and ZFHX3 (involved in atrial fibrillation and cardioembolic stroke).		
miR-5196-5p	Targets SH3PXD2A gene.		
miR-4292	Targets CASZ1 and FURIN genes that are involved in blood pressure and the STXBP5 gene.		
miR-1268a, **miR-1268b,** **miR-4433b-3p,** **miR-6089,** **miR-6090,** **miR-6752-5p** **miR-6803-5p**	Target genes involved in the cellular nitrogen compound metabolic process, small molecule metabolic process, blood coagulation, cell–cell signaling, cell junction assembly, chondroitin sulfate metabolic process, glycosaminoglycan metabolic process, energy reserve metabolic process, response to stress, platelet activation, cell	Risk stratification and prediction	32

	junction organization, membrane organization, adherens junction organization, and cell adhesion.		

Table 3. Summary of miRNAs, their effect, and their potential use as biomarkers in intracranial hemorrhage.
miR, microRNA; NF-κB, nuclear factor-κB; TGF-β, transforming growth factor-beta; SRF, serum response factor; VSMC, vascular smooth muscle cell; JAK-STAT, Janus kinase-signal transducer and activator of transcription proteins; *ZO1*, zonula occludens-1; MAPK8, mitogen-activated protein kinase-8; NURR1, nuclear receptor related-1 protein; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin- 1 beta; IL-6, interleukin-6.

Appendix 1

Keywords	Database	Initial results	After screening	Eligible
"MicroRNAs," AND "spontaneous intracerebral hemorrhage," AND "hemorrhagic stroke" **MeSH terms: Micro RNAs OR "MicroRNAs/analysis"[Mesh] OR "MicroRNAs/blood"[Mesh] OR "MicroRNAs/cerebrospinal fluid"[Mesh] OR "MicroRNAs/cytology"[Mesh] OR "MicroRNAs/etiology"[Mesh] OR "MicroRNAs/genetics"[Mesh] OR "MicroRNAs/statistics and numerical data"[Mesh] OR "MicroRNAs/therapeutic use"[Mesh] AND Intracranial hemorrhage Or "Intracranial Hemorrhages/blood"[Mesh] OR "Intracranial Hemorrhages/cerebrospinal fluid"[Mesh] OR "Intracranial Hemorrhages/complications"[Mesh] OR "Intracranial Hemorrhages/diagnosis"[Mesh] OR "Intracranial Hemorrhages/diagnostic imaging"[Mesh] OR "Intracranial Hemorrhages/drug therapy"[Mesh] OR "Intracranial Hemorrhages/etiology"[Mesh] OR "Intracranial Hemorrhages/genetics"[Mesh] OR "Intracranial Hemorrhages/microbiology"[Mesh] OR "Intracranial Hemorrhages/pathology"[Mesh] OR "Intracranial Hemorrhages/physiopathology"[Mesh] OR "Intracranial Hemorrhages/prevention and control"[Mesh] OR "Intracranial Hemorrhages/therapy"[Mesh] OR hemorrhagic stroke OR "Hemorrhagic Stroke/analysis"[Mesh]**	PubMed	25,471	15	8

OR "Hemorrhagic Stroke/blood"[Mesh] OR "Hemorrhagic Stroke/diagnosis"[Mesh] OR "Hemorrhagic Stroke/etiology"[Mesh] OR "Hemorrhagic Stroke/genetics"[Mesh] OR "Hemorrhagic Stroke/physiology"[Mesh] OR "Hemorrhagic Stroke/physiopathology"[Mesh] OR "Hemorrhagic Stroke/prevention and control"[Mesh]				
"MicroRNAs" AND "Brain Hemorrhage"	Embase	358	3	2

Keywords used for the systematic search.

MeSH, Medical Subject Headings.