Circulating microRNAs as biomarkers for acute ischemic stroke: a systematic review

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Abstract

Acute ischemic stroke is a leading cause of death and disability worldwide. Unlike myocardial infarction there is no current blood test to diagnose acute ischemic stroke. MicroRNAs are very stable in the blood and have been suggested as potential diagnostic markers. This review aimed to systematically assess case-control studies investigating the association of circulating microRNAs with acute ischemic stroke. Medline, CINAHL, Cochrane Library, Web of Science Scopus, and PubMed were searched for studies that examined the association of circulating microRNAs in acute ischemic stroke patients. Studies meeting specific inclusion and exclusion criteria (such as blood sample were obtained within 24 hours of an acute ischemic stroke) were selected for data extraction. Two authors extracted data from the included studies relevant to study design, patient characteristics, and relative microRNA expression. Eight studies were included involving 572 cases and 431 healthy controls. Twenty-two microRNAs (12 up-regulated and 10 downregulated) were reported as differentially expressed. Only one microRNA, miR-106b, was reported as differentially expressed in at least 2 studies. Significant heterogeneity in the design and methods of the included studies was noted. Differential expression of a large number of microRNAs has been reported early following acute ischemic stroke. More research is required in larger patient populations to further evaluate the diagnostic potential of the reported microRNAs.

Introduction

Stroke is the second leading cause of death and third leading cause of disability worldwide with an incidence of 16.9 million cases globally in 2010 [1]. The main causes of ischemic stroke are large artery athero-thrombosis and cardiac embolism [2]. The main treatments for ischemic stroke are mechanical and chemical thrombolysis [3, 4], however these treatments need to be administered rapidly following the stroke to be effective [5]. Thrombolysis is also associated with a risk of bleeding, including intracranial hemorrhage [6]. Rapid and accurate diagnosis of acute ischemic stroke is therefore critical to enable appropriate treatment. Currently diagnosis of acute ischemic stroke relies on neuroimaging techniques such as magnetic resonance imaging (MRI) and computed tomography (CT) scans, however the availability of such expensive machinery can be limited, and image interpretations may be inconsistent during the early stages of stroke [7]. Therefore, there is a need for a more rapid and simple tool for acute ischemic stroke diagnosis. Myocardial infarction is routinely diagnosed using blood markers and it has been suggested that circulating biomarkers could also be valuable for acute ischemic stroke diagnosis. MicroRNAs (miRNAs) are small RNA molecules that may serve as valuable biomarkers due to their easy detection and stability in blood samples [8]. While many studies have investigated miRNA expression in acute ischemic stroke patients, there has been no previous systematic review of the diagnostic potential of miRNAs for acute ischemic stroke. This systematic review aimed to summarize previous research examining the association between circulating miRNAs and acute ischemic stroke in blood samples collected within 24 hours of stroke symptom onset. We focused on these studies as a circulating marker is needed in clinical practice for diagnosing acute ischemic stroke in presenting patients.

Materials and Methods

This systematic review was performed in line with the reporting guidelines of the systematic review and meta-analysis (PRISMA) statement [9]. A protocol was developed following the guidelines of the PRISMA-P statement [10] and was published in the PROSPERO database (CRD42016036218).

Search Strategy

A systematic literature search was conducted using Medline (via Ovid MEDLINE®, 1946 to February Week 4 of 2016), CINAHL, Cochrane Library, Web of Science (1965 to 2016), Scopus (1960-2016), and PubMed. Searches were performed with a combination of MeSH (medical subject headings) terms and keyword terms. Medline, CINAHL, and Cochrane Library were searched with MeSH terms “MicroRNAs” AND “Biomarkers OR Genetic Markers” AND “Stroke”. With aid from a librarian with specified skills for database searching, the following search string was developed and used in all of the chosen databases: "micro rna" OR microrna* OR mirna* OR "small temporal rna" OR strna AND "biochemical marker" OR "biochemical markers" OR "biologic marker" OR "biologic markers" OR "biological marker" OR "biological markers" OR biomarker* OR "clinical marker" OR "clinical markers" OR "immune marker" OR "immune markers" OR "immunologic marker" OR "immunologic markers" OR "laboratory marker" OR "laboratory markers" OR "serum marker" OR "serum markers" OR "surrogate end point" OR "surrogate end points" OR "surrogate endpoint" OR "surrogate endpoints" OR "surrogate marker" OR "surrogate markers" OR "viral marker" OR "viral markers" AND "cerebrovascular accident" OR "cerebrovascular accidents" OR stroke* OR apoplex* OR "brain vascular accident" OR "brain vascular accidents" OR cva* OR "cerebral

vascular accident" OR "cerebral vascular accidents" OR "brain infarction" OR "brain infarctions" OR "brain venous infarction" OR "brain venous infarctions".

Study Selection

The studies included in this review were selected based on the following inclusion and exclusion criteria.

Studies were included if:

1. They had a case-control design;
2. The cases included acute ischemic stroke patients who were evaluated by neuroimaging;
3. The controls were healthy participants;
4. Blood samples were collected within 24 hours of stroke symptom onset, enabling identification of miRNAs which rapidly change immediately after stroke;
5. They evaluated the expression level of circulating miRNAs in blood samples from both cases and controls.

Studies were excluded if:

1. They investigated miRNA levels solely in animal models;
2. They had a total case population of less than 50 persons;
3. The article was not available in English.

Studies were still included if a subset of the included participants met the inclusion criteria, as long as miRNA data could be extracted for the subset of individuals that satisfied the inclusion criteria. The titles and abstracts of articles obtained from the search strategy were scanned to identify unique eligible studies. Eligible studies were selected for inclusion after a full

text analysis. A further search was performed within the reference lists of studies and reviews obtained from the database search.

Data Extraction

Two people (BD and DM) independently performed data extraction using a pre-determined data extraction form (S1 Table). This form was designed to collect information regarding general patient characteristics, including stroke associated risk factors, case and control definitions, methods of miRNA quantification, and expression levels of circulating miRNAs. If data were not provided in the article or supplementary information, the corresponding author was contacted to request the missing information. miRNA data that was not numerically stated and not supplied after contacting authors was extrapolated from published figures using Adobe Photoshop CC (2015). A consensus meeting was held between the two reviewers to resolve any differences.

Quality Assessment

The quality of the included studies were assessed using a modified QUADAS 2 tool (S2 Table) [11]. This tool was modified to include questions specific to miRNA research and acute ischemic stroke. Questions evaluated the reported definitions and diagnoses of stroke, the methods of miRNA evaluation, and the reporting of confounding risk factors. The questions were answered with 'Yes', 'No', or 'Unclear' and scored as per the evidence based librarianship critical appraisal tool [12]. Two authors (BD and DM) independently and blindly scored the studies as the total number of 'Yes' responses. Any discrepancies of 2 or more points were discussed in a consensus meeting and conflicts were resolved. Studies were deemed as high

quality, moderate quality, or low quality if the average quality assessment scores were ≥75%, 50-75%, or ≤50%, respectively.

Results

Literature Search

The initial search of the databases yielded 339 papers eligible for inclusion. After 187 duplicates were removed, 152 unique abstracts were screened for eligibility. Of these, 132 were excluded due to not meeting the inclusion criteria, or they were review papers. The full texts of the remaining 20 studies were assessed. Twelve of these studies were excluded, mainly due to blood samples not being obtained within 24 hours of stroke onset, thereby prohibiting assessment of changes in miRNA expression related to stroke onset and/or assessment of patient populations <50 persons. A total of 8 studies satisfied the inclusion and exclusion criteria and were included in this review (Figure 1) [13-20].

Fig 1. Outline of the literature search and study selection process. No additional studies were added after searching through reference lists of publications identified during the search

Quality Assessment

The quality assessment suggested that two studies were of high quality [15, 18], four studies of moderate quality [16, 17, 19, 20], and two studies of low quality [13, 14] (S2 Table). Only two of the studies adjusted for confounding risk factors of stroke, one of which received the highest quality score [15] and the other receiving the lowest quality score [14]. The two studies with high quality scores successfully defined their controls as having no prior history of stroke,

reported on stroke risk factors for patients, and avoided pooling blood samples for miRNA detection [15, 18]. In contrast, the two studies with low quality scores did not meet these criteria and failed to report p-values for the miRNAs that were reported to be differentially expressed [13, 14]. The study receiving the lowest quality score was the only study in which controls were not age and sex matched to the acute ischemic stroke cases[14].

Study Design and Methods

The design and methods of the included studies are summarized in Table 1. Seven of the studies were performed in China [13, 15-19] and one in Singapore [14]. Three studies diagnosed acute ischemic stroke based on clinical signs of neurological deficit [15-17] and four diagnosed acute ischemic stroke based on cerebral infarction using MRI and/or CT [13, 14, 19]. One study stated patients were assessed for neurological deficit and examined by MRI with diffusion weighted imaging (DWI), however, it was unclear which method was used for diagnosis of acute ischemic stroke [18]. The definitions of controls varied between the studies as either having no physical evidence of stroke [13, 15], no prior history of stroke [15, 18], or having no stroke associated risk factors [19]. Four of the studies had control groups that were not clearly defined [14, 16, 17, 20]. The majority of the studies assessed the plasma [13, 15, 16, 20] and serum [17-19] from the blood samples, and one study assessed whole blood samples [14]. In three studies primary screening of blood samples was performed with a microarray chip followed by verification of microarray data using quantitative reverse transcription-Polymerase Chain Reaction (qRT-PCR) [13, 14, 17]. The remaining studies investigated specific miRNAs based on previous research [15, 16, 19]. All studies quantified differentially expressed miRNAs directly from blood samples by qRT-PCR using various housekeeping genes [13-20]

184 Table 1. Study design and methodology of the included studies.

Reference	Country	Definition of stroke	Definition of control	Sample type	Primary screening/validation †	miRNA quantification
[13]	China	Radiographic diagnosis	No evidence of stroke	Plasma	•Agilent miRNA microarray (1347 miRNAs) •pooled samples (n=76 AIS) •miRNAs validated with qRT-PCR assay (n=76 AIS)	•RT and qPCR •RNU6B control •relative expression via 2-ΔΔCt
[14]	Singapore	Radiographic diagnosis	Unclear	Whole blood	•miRCURY LNA array •individual and pooled samples (n=68 AIS) •10 random miRNAs validated with qPCR (n=169 AIS)	•RT and qPCR •RNU44 control •method for quantification not stated
[15]	China	Clinical diagnosis and radiographic diagnosis	No evidence of stroke and no prior history of cerebrovascular disease	Plasma	N/A	•RT and qPCR •U6 control •relative expression via 2-ΔΔCt
[16]	China	Clinical diagnosis and radiographic diagnosis	Unclear	Plasma	N/A	•RT and qPCR •synthetic RNA oligonucleotide from each miRNA sequence •miRNA copy numbers calculated via standard curve
[17]	China	Clinical diagnosis and radiographic diagnosis	Unclear	Serum	•miRCURY LNA Array (3100 miRNAs) •pooled samples (n=40 AIS) •miRNAs validated with qRT-PCR (n= 22 AIS)	•RT and qPCR •syn-cel-lin-39 control •relative expression via 2-ΔΔCt
[18]	China	Unclear	No prior history of cerebrovascular disease	Serum	•primary screening of 9 miRNAs by qRT-PCR (n=30 AIS)	•RT and qPCR •U6 control •relative expression via 2-ΔΔCt
[19]	China	Radiographic diagnosis	No cerebrovascular risk factors	Serum	N/A	• RT and qPCR •18S rRNA control •relative expression via 2-ΔΔCt
[20]	China	Clinical diagnosis and radiographic diagnosis	Unclear	Plasma	N/A	• RT and qPCR •cel-mir-39 •relative expression via 2 exp (mean Ct spiked-in controls - Ct target miRNA) and log transformed

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AIS, acute ischemic stroke; RT, reverse transcription; qPCR, quantitative polymerase chain reaction. N/A, not applicable

† Primary screening methods and validation of primary screening may have included a separate patient population not specific to those analyzed within 24 hours; *n* number of patients used in each phase is state

Patient Characteristics

A summary of the characteristics of the cases and controls included in the 8 studies are presented in Table 2. In studies where only a sub-set of patients met the inclusion criteria the characteristics of these patients are reported unless otherwise stated. The acute ischemic stroke case number ranged from 38 to 146 [15, 18], with the mean age of patients ranging from 50 to 72 [13, 19]. In all of the studies at least 50% of the cases were male (range 50% to 72.4%). Six out of the eight studies reported stroke associated risk factors with a large percentage of the cases having hypertension [14-16, 18, 19]. The risk factors in controls varied from having no risk factors [14, 19, 20], to greatly reduced prevalence of risk factors compared to cases [15], or similar prevalence to the cases [18]. Three studies did not report the risk factors for controls [13, 16, 17]. An attempt was made to contact the corresponding authors to obtain missing data however none replied.

Table 2. Characteristics of acute ischemic stroke patients and healthy controls included in this systematic review.

Reference	AIS Cases							Healthy Control						
	n*	Age	M%	HT%	DM%	HL%	S%	n*	Age	M%	HT%	DM%	HL%	S%
[13]	76	50 ± 13 †	55.1	ns	ns	ns	ns	116	53 ± 11	51.7	ns	ns	ns	ns
[14]	45	59.7 ± 1.39 †	61.4†	77.6†	44.6†	75.3†	ns	24	39.0 ± 8.10	75	0	0	0	ns
[15]	38	62.4 ± 5.9 ‡	50	18.4	13.1	15.8	18.4	50	64 ± 6	48	10	10	12	20
[16]	74	72 [18] 43-92 †§	50.5†	74.2†	34.4†	18.3†	15.1†	23	65 §	69.6	ns	ns	ns	ns
[17]	53	68 ± 10.9	58.5	ns	ns	ns	ns	50	67 ± 8.5	58.0	ns	ns	ns	ns
[18]	146	67.29 ± 14.16	76.7	86.3	39.1	74.6	44.5	96	63.23 ± 15.24	66.7	72.9	34.3	35.4	43.7
[19]	72	72.4 ± 9.2	56.9	31.9	23.6	26.4	22.2	51	70.7 ± 7.5	58.8	0	0	0	17.6
[20]	68	64 (55-76) §	66.2	54.4	25.0	11.7	26.5	21	58 (54-67) §	52.6	0	0	0	0

AIS, acute ischemic stroke; n, number of acute ischemic stroke patients or healthy controls; M%, male percentage of the population; HT%, percentage of population with hypertension; DM%, percentage of population with Diabetes Mellitus; HL%, percentage of population with hyperlipidemia; S%, percentage of current smokers; ns, data not stated; N/A, applicable. Age is stated in years (mean ± SD) unless otherwise specified.

* n number of acute stroke patients in the study who had blood sample collection within 24 hours and who were involved in the analysis producing the extractable miRNA data (Table 3), or the number of controls involved in the analysis.

† Representative of a larger patient population used in the study and is not specific to reported 'n' population

‡ Data calculated as pooled mean and standard deviation from ages of subgroups

§ Data expressed as medians [interquartile range] range.

Circulating miRNAs and their relative expression

The extracted miRNA data includes differentially expressed miRNAs quantified directly from blood samples that were collected within 24 hours of stroke. This excluded data from primary screening to avoid pooled samples. The miRNAs identified as differentially expressed in the blood of acute ischemic stroke patients varied greatly amongst the studies and are summarized in Table 3. A total of 22 miRNAs were reported as differentially expressed across all studies with 12 reported to be upregulated and 10 reported to be downregulated in stroke cases relative to healthy controls. Six of the 22 miRNAs (let-7b, miR-16, miR-21, miR-106b, miR-320d and miR-1246) were identified during primary screening in other included studies [13, 14] however were not selected for further quantification in these studies for reasons that were not always made clear and thus were not reported in this review. miR-106b was the only miRNA reported to be differentially expressed in a common direction in more than one study [13, 17]. Two studies reported significant up-regulation of miR-106b in the blood of acute ischemic stroke patients compared to healthy controls, although the relative expression of this miRNA varied greatly (1.46-fold up-regulation [17] versus 23.9-fold up-regulation [13]). Two members of the let-7 family were reported to be associated with acute ischemic stroke. In one study let-7b was reported as downregulated (0.208-fold) in patients with large artery atherosclerosis stroke but upregulated (9.45-fold) in patients with cardioembolic stroke [15]. In another study let-7e was reported to be 1.77-fold upregulated in acute ischemic stroke patients compared to healthy controls [19]. Two members of the miR-320 family (miR-320d and miR-320e) were reported as downregulated in acute ischemic stroke patients (Table 3) [13]. The other 17 miRNAs reported as differentially expressed in acute ischemic stroke patients were identified in single studies and varied in expression relative to healthy controls (Table 3).

Table 3. miRNAs reported to be significantly differentially expressed in blood samples obtained within 24 hours of onset of acute ischemic stroke.

miRNA	Relative expression*	Up/down regulated	p-value	ROC analysis: AUC (95%CI); p-value; *n*
let-7b	0.209; 9.36 [15] †	Downregulated; upregulated†	<0.05; <0.05	0.93 (0.879-0.980); p-value ns; n=38
let-7e	1.77 [19]	Upregulated	0.0091	0.86 (0.754-0.968); p-value ns; n=72
miR-16	1.33 [16]	Upregulated	0.0088	N/A
miR-21	0.252 [20]	Downregulated	<0.05	N/A
miR-23a	0.43 [18]	Downregulated	<0.01	N/A
miR-24	0.287 [20]	Downregulated	<0.05	N/A
miR-27a	7.28 [14]	Upregulated	ns	0.88 (0.81-0.96); p-value ns; n=101 ‡
miR-30a	0.288; 0.266 [15] †	Downregulated; downregulated†	<0.05; <0.05	0.91 (0.869-0.979); p-value ns; n=38
miR-32-3p	1.58 [17]	Upregulated	<0.05	N/A
miR-106b-5p	23.90 [13] 1.46 [17]	Upregulated Upregulated	ns <0.05	0.962 (0.930-0.993); p =0.000; n=76 N/A
miR-125b-2	2.75 [14]	Upregulated	ns	0.85 (0.77-0.93); p-value ns; n=101‡
miR-126	0.0505; 0.0505 [15] †	Downregulated; downregulated†	<0.05; <0.05	0.92 (0.871-0.978); p-value ns; n=38
miR-145	7.72 [18]	Upregulated	<0.001	N/A
miR-221	0.154 [18]	Downregulated	<0.001	N/A
miR-320d	0.07 [13]	Downregulated	ns	0.987 (0.972-1.000); p=0.000; n=76
miR-320e	0.13 [13]	Downregulated	ns	0.981 (0.963-0.998); p=0.000; n=76
miR-422a	2.63 [14]	Upregulated	ns	0.86 (0.75-0.97); p-value ns; n=101 ‡
miR-488	2.77 [14]	Upregulated	ns	0.86 (0.72-0.92); p-value ns; n=101 ‡
miR-532-5p	0.696 [17]	Downregulated	<0.01	N/A
miR-627	5.50 [14]	Upregulated	ns	0.76 (0.66-0.87); p-value ns; n=101‡
miR-1246	1.95 [17]	Upregulated	<0.05	N/A
miR-4306	5.30 [13]	Upregulated	ns	0.952 (0.922-0.982); p =0.000; n=76

ROC, receiver operator characteristic; AUC, area under the curve; ns, not stated; N/A, not applicable. p-values are reported from the included studies for miRNA expression differences in cases versus controls.

*Relative expression given as a ratio to healthy control group. Relative ratios were either obtained directly from the article or were calculated using miRNA expression levels from cases and controls to generate a ratio.

† The relative expression in patients from TOAST classification subgroups with large vessel atherosclerosis (n=10) and cardioembolism (n=9), respectively. miRNA levels were also reported in the study for other subgroups [15].

‡ ROC analysis included a larger patient population not specific to those analyzed within 24 hours; *n* number of patients used in the analysis is stated.

Assessment of the diagnostic potential of circulating microRNAs for acute ischemic stroke

Four of the studies performed receiver operator characteristic (ROC) analyses to examine the diagnostic potential of the differentially expressed miRNAs [13-15, 19]. The highest area under the curve (AUC) value was reported for miR-320d (0.987) [13] while the lowest AUC value (0.76) was reported for miR-627 (Table 3) [14]. Only one of the two studies that associated miR-106b with acute ischemic stroke performed a ROC analysis and reported an AUC of 0.962 [13]. Let-7b, miR-126, miR-30a, miR-320e, and miR-4306 were each reported to have AUC values greater than 0.90 [13, 15].

Discussion

Following a systematic literature search, we identified 22 circulating miRNAs reported to have an association with acute ischemic stroke. Of these, only miR-106b was consistently reported as significantly upregulated in more than one study, however the relative expression varied substantially between the 2 studies [13, 17]. These findings contradicted those of another included study that reported down-regulation of miR-106b in acute ischemic stroke patients [14]. Sepramaniam and colleagues reported down-regulation of miR-106b and let-7b in both acute ischemic stroke and stroke recovery patients, thus were not selected for further assessment in their study due to not being unique to acute ischemic stroke [14]. Similarily, the down-regulation of miR-21 reported in this review was contradicted by that of another included study, which demonstrated differential up-regulation of miR-21 during primary screening [13]. Wang and colleagues did not select miR-21 for further assessment in their study [13] for reasons that

remain unclear. Other included studies demonstrated miR-16, miR-320d and miR-1246 to be differentially expressed in acute ischemic stroke patients in a similar direction to findings observed in this review, however, they were not selected for further assessment in these studies for reasons that were not clear [13, 14].

Multiple factors may have contributed to the observed differences in miRNA expression between the studies. Firstly, the participant characteristics varied between the studies. The controls varied from those with similar risk factors to the cases to those with no risk factors [14, 15, 19, 20]. Two of the studies did not report stroke risk factors for the ischemic stroke patients [13, 17]; therefore it is unclear how these unknown variables may have affected the reported results. Secondly, the sample sizes of stroke patients and controls also varied substantially both between the studies and between each miRNA identification phase (primary screening, validation, and miRNA quantification). Thirdly, the methods of miRNA assessment varied between the studies. The type of blood medium analyzed varied across the studies and likely effected the relative concentrations of reported miRNAs [13-20]. Additionally, three of the studies employed microarray chips for primary identification of miRNAs with varying rationale for selection of miRNAs for further assessment [13, 14, 17], while other studies assessed miRNAs based on past research [15, 16, 18-20]. Across all the studies a number of different housekeeping genes were used during miRNA quantification, which may have effected the normalization of miRNA expression. Finally, different classifications of stroke within the included patients may have contributed to the variations in miRNA expression. Large artery-atherosclerotic stroke and cardioembolic stroke occur by different pathological mechanisms and this may affect the relative expression of some miRNAs. This is supported with data from one

303 included study that reported a substantial difference in let-7b expression for large artery-
304 atherosclerotic stroke and cardioembolic stroke [15].

305 The diagnostic potential of the reported miRNAs is difficult to assess since only 4 of the
306 included studies performed ROC analysis with varying outcomes. Of the 13 miRNAs reported
307 with AUC values let-7b, miR-30a, miR-106b, miR-126, miR-320d, miR-320e, and miR-4306
308 were reported to have AUC values greater than 0.90. A previous study identified differential
309 expression of these miRNAs in brain and blood samples of a rat ischemic stroke model [21].
310 However, of these only miR-320 was identified as both differentially expressed in brain and
311 blood samples of the rat model [21], suggesting they were representative of the acute brain
312 response to stroke. This is consistent with findings from this review where miR-320d and miR-
313 320e were reported to have the highest AUC values amongst the 20 miRNAs. More research is
314 required on the miRNAs identified in this review to further assess their potential as biomarkers
315 for acute ischemic stroke.

316 The current review has a number of limitations. Firstly only 8 studies met our inclusion
317 criteria and only 2 were considered to be of high quality. This suggests that the potential for
318 miRNAs to diagnose stroke remains poorly investigated, and there is a need for additional high-
319 quality studies to more thoroughly assess their clinical usefulness. Secondly, there is substantial
320 heterogeneity in the reported results from the included studies. The large variation in the reported
321 miRNAs makes the applicability of these findings for future diagnostics difficult to determine.
322 Thirdly, due to only including patients with blood samples collected within 24 hours, several
323 studies were excluded. We used this approach to focus on diagnostic markers that could be used
324 early after stroke, which is the current clinical requirement. Finally, the designs of the included
325 studies had inherent limitations. For example, all of the studies included Asian populations so it

is unclear whether these results are representative of other ethnic groups. The sample sizes of the included studies were relatively small. Additionally, most of the studies did not adjust for confounding risk factors. Therefore, interpretation of the results presented in this review should be made cautiously.

Conclusion

In conclusion this systematic review reports a large number of circulating miRNAs to be differentially expressed in acute ischemic stroke patients in blood samples collected within 24 hours of symptom onset. Currently miR-106b is the only miRNA to have been reported as differentially expressed in patients within 24 hours of ischemic stroke in more than one study. The highest AUC were reported for miR-320d and miR-320e and therefore these may have the best diagnostic potential although this remains to be confirmed in larger studies. Further studies employing larger and more diverse populations with better adjustment for confounding risk factors are required to determine the clinical value of miRNAs as biomarkers for acute ischemic stroke.

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Supporting Information

S1 Table. Study design of the included studies

S2 Table. Quality assessment of the studies

S1 File. PRISMA checklist

S2 File. PRISMA flow diagram