Circulating Non-coding RNAs as Potential Biomarkers for Ischemic Stroke: A Systematic Review

Jingjing Zhao

The First Affiliated Hospital of China Medical University

Qianwen Wang

The First Affiliated Hospital of China Medical University

Rui-xia Zhu (✉ zrx_200626313@163.com)

The First Affiliated Hospital of China Medical University <https://orcid.org/0000-0002-2683-4674>

Jun Yang

The First Hospital of China Medical University: The First Affiliated Hospital of China Medical University

Research Article

Keywords: ischemic stroke, miRNAs, lncRNAs, diagnosis, biomarker

Posted Date: January 18th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1244517/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background

Recent studies have demonstrated that dysregulated non-coding RNAs (ncRNAs) are involved in the pathogenesis of ischemic stroke (IS) including neuroinflammation, apoptosis, atherosclerosis and angiogenesis. However, discrepant results make it difficult to apply ncRNAs into clinical practice. Therefore, we performed this meta-analysis to evaluate and elucidate the diagnostic value of ncRNAs in IS.

Methods

We searched the literature in four databases: PubMed, Web of Science, EMBASE and the Cochrane Library up to December 31, 2020 to identify the relationship between differently expressed ncRNAs and IS. Pooled sensitivity, specificity, positive likelihood ratios (PLR), negative likelihood ratios (NLR), diagnostic odds ratio (DOR), and corresponding 95% confidence intervals (95%CI) were calculated to assess diagnostic performance of ncRNAs.

Results

15 studies for miRNAs containing 1687 IS patients and 8 studies for lncRNAs, including 741 IS patients were included in our study. Estimates of the identification efficiency of miRNAs from the 15 studies were: 0.76 (95%CI:0.67-0.84) for SEN, 0.83 (95%CI:0.77-0.88) for SPE, 4.5 (95%CI:3.4-6.1) for PLR, 0.29 (95%CI:0.20-0.40) for NLR, 16 (95%CI:10-26) for DOR. The AUC of the SROC curve showed diagnostic accuracy was 0.87 (95%CI:0.84-0.90), thus the diagnostic value of all the miRNAs was moderate. In addition, the results of lncRNAs were: 0.73 (95%CI: 0.69-0.77) for SEN, 0.75 (95%CI: 0.70-0.79) for SPE, 2.9 (95%CI: 2.4-3.4) for PLR, 0.36 (95%CI: 0.31-0.42) for NLR, 8 (95%CI: 6-11) for DOR, 0.80 (95%CI: 0.76-0.83) for AUC, also a moderate diagnostic value.

Conclusion

Our study revealed that blood circulating ncRNAs could be a moderately effective candidate biomarker for the diagnosis of IS. Furthermore, combined lncRNAs showed more accurate diagnostic properties than single lncRNAs and some single miRNAs (e.g. miR-107) showed better diagnostic performance, which may contribute to IS clinical practice.

1. Introduction

Stroke is a prevalent disease worldwide, with high disability and mortality. It has caused serious sociodemographic and socioeconomic burden in recent years, and the burden continues to increase (1). Ischemic stroke (IS) is a major subtype of stroke, for which thrombolysis (through the administration of tissue plasminogen), and thrombectomy (the surgical removal of emboli), are the main therapeutic methods. However, the number of patients benefiting from these therapies is relatively low

because of the narrow therapeutic window, hemorrhagic transformation, and reperfusion injury. As a medical emergency with high morbidity, there is a lack of serum biomarkers for the diagnosis and prevention of IS (2, 3). Recent studies have found that non-coding RNAs (ncRNAs) show differential expression during IS and play widespread roles in the different phases of the disease (4-6).

Recent studies have demonstrated that ncRNAs are involved in the pathogenesis of IS, including neuroinflammation, apoptosis, atherosclerosis, and angiogenesis (5,6). NcRNAs are defined as non-protein-coding transcripts and are classified into many types according to their length and connection, e.g. microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs. lncRNAs are a type of linear RNA over 200 nucleotides in length, whereas miRNAs are 22 nucleotide long ncRNAs (7). As major members of the ncRNA family, lncRNAs and miRNAs often exhibit cell and tissue specificity. They also play extensive roles in pathophysiological events by regulating the expression of mRNA and protein (8-10). MiRNAs regulate gene expression at the post-transcriptional level by binding to the 3 prime untranslated region of mRNA, whereas lncRNAs exert their effects at the transcriptional and post-transcriptional levels (7, 11). Recently, emerging studies have begun to reveal that ncRNAs can accumulate and be expressed in mammalian brain and in neuronal cell lines and play several roles in physiological and pathological activities in the brain (12).

To date, multiple dysregulated miRNAs and lncRNAs (upregulated or downregulated) have been identified from the middle cerebral artery occlusion model (MACO), the oxygen glucose deprivation/re-oxygenation cell model (OGD/R), and from the blood of IS patients. Stroke significantly alters the expression profile of miRNAs and lncRNAs, indicating that these ncRNAs have the potential to be diagnostic and predictive biomarkers for IS (13-16). However, discrepant reports make it difficult to apply ncRNAs into clinical practice. Moreover, not all ncRNAs are suitable as potential biomarkers for IS. The diagnostic potential of ncRNAs is yet to be determined; we performed this meta-analysis to evaluate and elucidate the diagnostic value of ncRNAs in IS.

2. Material And Methods

2.1 Search Strategy

We systematically searched the literature in four databases: PubMed, Web of Science, EMBASE, and the Cochrane Library, up to December 31, 2020. The literature on ncRNAs was retrieved using the following search strategies: (microRNA OR miRNA OR long non-coding RNA OR lncRNA OR non-coding RNA OR ncRNA) AND (diagnosis OR specificity OR sensitivity OR receiver operating characteristics OR ROC) AND (ischemic stroke OR cerebral infarction OR brain infarction OR cerebrovascular disease). We also checked references in these articles to identify further relevant studies. All studies identified were inspected by two independent reviewers, and disagreements, if any, were discussed until a consensus was reached.

2.2 Inclusion Criteria

The eligible studies should meet the following criteria: (1) Studies were focused on the diagnostic performance of lncRNAs or miRNAs in IS; (2) Describing peripheral blood detection (plasma, serum, and whole blood); (3) All the patients were diagnosed as IS according to radiological imaging (CT or MRI) and neurological examination; (4) The sufficient data including the sample size of case and controls, sensitivity (SEN), specificity (SPE) and area under curve (AUC) were available. In addition, the exclusion criteria are as follows: (1) Literature not written in English; (2) Reviews, meeting records or letters; (3) Studies not conducted in humans; (4) Not describing peripheral blood detection of lncRNAs and miRNAs.

2.3 Data Extraction and Quality Assessment

Two researchers extracted information from included literature covering first author, publication year, ethnicity, method, sample size of case and control, sample source, and necessary data including SPE, SEN and AUC. Quality assessment of these studies were carried out by two independent reviewers according to the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) (17), which contain 4 domains: patient selection, index test, reference standard, and flow and timing. Each domain is used for bias assessment and the first 3 domains were applied to evaluate clinic applicability of these studies.

Data availability statement All data generated or analyzed during this study are included in this published article (and its supplementary information files).

2.4 Statistical Analysis

The extracted data were analyzed using STATA 15.0 and $p < 0.05$ was considered statistically significant. Pooled sensitivity (SEN), specificity (SPE), positive likelihood ratios (PLR), negative likelihood ratios (NLR), diagnostic odds ratios (DOR), and corresponding 95% confidence intervals (95% CIs) were calculated using a random effects model. In addition, the summary receiver operator characteristic (SROC) curve and the area under the SROC curve (AUC) were analyzed to assess the diagnostic performance of lncRNAs and miRNAs for IS. A summary of the PLR and NLR, as well as the Fagan's nomogram, were used to evaluate the clinical applicability of ncRNAs for the diagnosis of IS. Cochran's-Q and I-squared (I^2) tests were applied for assessment of heterogeneity, with $p < 0.1$ or $I^2 > 50\%$ indicative of significant heterogeneity. ROC plane, sensitivity analysis, bivariate boxplot, and meta-regression were further conducted to explore the potential sources of heterogeneity. A group of analysis with a goodness-of-fit, a bivariate normality, a influence analysis and a outlier detection were used to validate our results. A funnel plot was used to examine publication bias.

3. Results

3.1 Study Characteristics

We searched 1458 articles in total from PubMed (904), Web of Science (389), EMBASE (152), and the Cochrane Library (13), with 634 and 824 articles for miRNAs and lncRNAs, respectively. Of these,

1364 records (566 and 798 for miRNAs and lncRNAs, respectively) were excluded after reading the title and abstract due to: duplication; not describing miRNAs or lncRNAs with IS; not a human model; not describing peripheral blood detection; or studies based on databases. Subsequently, 94 articles remained (66 and 28 for miRNAs and lncRNAs, respectively). After reading the whole text, 71 articles were excluded because they did not provide the necessary data, leaving 23 articles ultimately included for miRNAs and lncRNAs (18-40). A flowchart of the whole selection process is shown in Figure 1.

Of these remaining articles, 15 were miRNA studies and included 1687 IS patients and 1267 controls. There were 27 sets of miRNAs in total, from which single and combined miRNAs (double, triple, and pentuple) were described, in terms of their diagnostic value for IS, respectively in 20 and 7 (3 sets of double, 2 sets of triple, and 2 sets of pentuple miRNAs) studies (Table 1). Meanwhile, 8 studies were conducted for lncRNAs, encompassing 741 qualifying patients and 774 corresponding controls. There were 18 sets of lncRNAs performed in total, from which single and combined lncRNAs (double and triple) were described in 13 and 5 (3 sets of double and 2 sets of triple lncRNAs) studies respectively (Table 2).

3.2 Diagnostic Value of NcRNAs

3.2.1 Diagnostic Value of miRNA

The specificity and sensitivity of miRNAs in the diagnosis of IS are shown in forest plots of the 27 sets of miRNAs from the 15 selected studies (Figure 2). These plots indicate significant heterogeneity, with I^2 values of 95.74 and 89.43 for sensitivity and specificity, respectively, as such we used a random effects model in this meta-analysis. Estimates of the identification efficiency of miRNAs from 15 studies were 0.76 (95% CI: 0.67–0.84) for SEN, 0.83 (95% CI: 0.77–0.88) for SPE, 4.5 (95% CI: 3.4–6.1) for PLR, 0.29 (95% CI: 0.20–0.40) for NLR, 16 (95% CI: 10–26) for DOR. In addition, the AUC of the SROC curve, indicating diagnostic accuracy, was 0.87 (95% CI: 0.84–0.90); thus the diagnostic value of all the miRNAs was moderate (Figure 3). In subgroup analysis based on miRNA profile, estimates for the combined miRNAs and single miRNAs were 0.71 (95% CI: 0.53–0.83) and 0.78 (95% CI: 0.67–0.86) for SEN, 0.85 (95% CI: 0.71–0.93) and 0.83 (95% CI: 0.75–0.88) for SPE, 4.8 (95% CI: 2.8–8.2) and 4.5 (95% CI: 3.1–6.4) for PLR, 0.35 (95% CI: 0.23–0.53) and 0.26 (95% CI: 0.17–0.41) for NLR, 14 (95% CI: 9–21) and 17 (95% CI: 9–32) for DOR. In the ROC curve, single miRNA and combined miRNA groups had similar diagnostic values, with AUCs of 0.88 (95% CI: 0.84–0.90) versus 0.86 (95% CI: 0.82–0.88), respectively (Figure S1 and Figure S2 in supplementary materials). In summary of the PLR and NLR, the 22nd set of miRNA (miR-107) from the study by Yang et al. (29) had a PLR >10 and NLR <0.1 (figure 4a). Fagan's nomogram indicated that the positive post-test probability of diagnosing IS would increase to 53%, whereas the negative post-test probability of diagnosing IS would decrease to 7%, with a pretest probability of 20% (Figure 4b).

3.2.2 Diagnostic Value of lncRNA

Similarly, statistics about lncRNAs which were extracted from 8 selected studies were analyzed in the same way. As showed in forest plots (figure 5), significant heterogeneity was found, as I-square value

were 56.69 and 74.97 for sensitivity and specificity respectively. The result of lncRNAs were 0.73 (95%CI: 0.69-0.77) for SEN, 0.75 (95%CI: 0.70-0.79) for SPE, 2.9 (95%CI: 2.4-3.4) for PLR, 0.36 (95%CI: 0.31-0.42) for NLR, 8 (95%CI: 6-11) for DOR, 0.80 (95%CI: 0.76-0.83) for AUC (Figure 6). In subgroup analysis based on lncRNA profile, estimates of combined lncRNAs and single lncRNAs were: 0.77 (95%CI: 0.71-0.82) and 0.71 (95%CI: 0.66-0.76) for SEN, 0.75 (95%CI: 0.69-0.80) and 0.75 (95%CI: 0.69-0.80) for SPE, 3.0 (95%CI: 2.4-3.8) and 2.8 (95%CI: 2.2-3.6) for PLR, 0.31 (95%CI: 0.24-0.39) and 0.38 (95%CI: 0.32-0.46) for NLR, 10 (95%CI: 6-15) and 7 (95%CI: 5-11) for DOR. Compared with single lncRNA, combined lncRNA has a higher diagnostic accuracy, with a AUC of 0.83 (95%CI: 0.79-0.86) versus 0.79 (95%CI: 0.75-0.82) as showed in figure S3 and figure S4 (supplementary materials). In summary of the PLR and NLR, no study had a PLR >10 and NLR <0.1, and only the 15th set of lncRNA (lncRNA-[AC136007.2](#)) conducted by Li et al. (37) fell on the border line (Figure 7a). In Fagan's nomogram, the pretest probability was 20%, the PLR and NLR were 3 and 0.36 respectively, and the positive post-test probability and the negative post-test probability of lncRNAs would be 42% and 8% respectively (Figure 7b).

3.3 Sensitivity Analysis, Meta-regression Analysis, and Publication Bias

Significant heterogeneity was observed in the 15 miRNA studies, as shown by the forest plots (SEN: $I^2=95.74$, SPE: $I^2=89.43$). To find the potential source of this heterogeneity, we carried out ROC plane, sensitivity analysis, bivariate boxplot, and meta-regression analysis. In the ROC plane, no typical shoulder arm was observed, indicating that no significant heterogeneity was caused by the threshold effect (Figure 8a). Influence analysis showed the study conducted by Liu et al. (23) may have influence on heterogeneity (Figure 8c). The bivariate boxplot revealed that 4 studies (Liu 2019, Lu 2018, Peng 2015 and Zeng 2011) contributed to this high heterogeneity (Figure 8d). After excluding these 4 studies, the pooled estimates did not change, whereas the sensitivity and specificity heterogeneity decreased (Figure S5). To confirm this result, we conducted further analysis for heterogeneity using a goodness-of-fit, bivariate normality, influence analysis and outlier detection, and found similar results (Figure S6). We read these 4 studies again and carried out further meta-regression analysis on the bias of sample size (≤ 50 or >50), sample source (serum, plasma or whole blood) and publication year (in 5 years or not). And we found that sample size and publication year may accounted for part of heterogeneity, with respectively $p < 0.01$ and $p < 0.05$ for sensitivity and specificity in sample size, and $p < 0.01$ for specificity in publication year (Figure 8b). Publication bias existed across the included studies on miRNAs, as identified by Deek's funnel plot ($p < 0.05$) (Figure 9).

As for lncRNAs, significant heterogeneity exist among the 8 lncRNA studies in forest plots (SEN: $I^2=56.69$, SPE: $I^2=74.97$) (Figure 5). Similarly, ROC plane, sensitivity analysis, bivariate boxplot, and meta-regression analysis were carried out. ROC plane showed the significant heterogeneity did not result from threshold effect (Figure 10a). Influence analysis showed no study exert in substantial role in the results (Figure 10c). Three studies (Li 2019, Wang 2017, and Wang 2019) were out of the boxplot and may be the source of heterogeneity (Figure 10d). After excluding the 3 studies, the pooled estimates were similar to the previous results, whereas heterogeneity of sensitivity and specificity decreased (SEN: $I^2=27.71$, SPE: $I^2=29.26$) (Figure S7). Subsequently, a further analysis with a goodness-of-fit, a bivariate normality, a

influence analysis and a outlier detection for heterogeneity showed the same result (Figure S8). Other source of heterogeneity indicated by meta-regression analysis may be sample size (>50 or not) with a $P < 0.01$ for sensitivity and a $P < 0.001$ for specificity (Figure 10b). No significant publication bias was found in Deek's funnel plot with a P of 0.91 (Figure 11).

4. Discussion

Several studies have reported aberrant expression of miRNAs and lncRNAs in IS patients (41-50). However, the results of these studies were inconsistent and have not yet reached an consensus. Some are significantly up-regulated, by contrast others are down-regulated in the circulating blood of IS patients. The discrepant results among these studies make it difficult to apply ncRNAs to clinical practice. Therefore, we reviewed 15 studies of miRNAs and 8 studies of lncRNAs and performed a systemic meta-analysis to clarify confusions about the diagnostic value of circulating ncRNAs, which would give suggestions for clinical applicability.

Our meta-analysis revealed a moderate diagnostic performance for blood-based miRNAs for IS. Single miRNAs and combined miRNAs showed no significant difference in diagnostic accuracy as measured by AUC in subgroup analysis (Figure S1 and Figure S2 in supplementary materials). Clinical applicability for diagnosis is dependent on the summary of PLR and NLR and Fagan's nomogram. In summary of the PLR and NLR, $PLR > 10$ and $NLR < 0.1$ represent for a higher diagnostic accuracy (51). Among the miRNAs, miR-107 from the study by Yang et al. (29) was in this scope (Figure 4a), hence miR-107 could be more beneficial in clinical diagnostics and deserves further research. In their study, circulating miR-107, miR-128b, and miR-153 are higher in IS patients than healthy controls, but miR-107 showed the greatest diagnostic value with the higher specificity and sensitivity. Yang et al. found miRNA-107 promoted excitatory neurotoxicity by regulating glutamate transporter-1 expression in I/R injury rat model (52). Additionally, the study by Cheng et al. has found that miRNA-107 participated in neuronal apoptosis process by p53 pathway in OGD-treated HT-22 hippocampal neuron cell (53). Recent study by Li et al. have demonstrated that miRNA-107 involved in angiogenesis after stroke by targeting Dice (45). In conclusion, miR-107 played an important role in the pathophysiological process of stroke. Hence, miR-107 is a novel candidate biomarker for the differentiation of IS patients from healthy individuals and needed further intensive study. Additionally, the diagnostic odds ratios of miRNAs was 16, which further confirmed miRNAs as a promising diagnostic tool for IS.

Multiple studies have reported that single lncRNAs possess good diagnostic performance for clinical use (34, 36-40). Whereas Deng et al. (33) found that the combination of linc-DHFRL1-4, SNHG15 and linc-FAM98A-3 showed greater diagnostic value with an AUC value of 0.842, and could precisely distinguish IS patients from healthy control. In our analysis, we summarized lncRNAs and identified that combined lncRNAs yield better diagnostic capability compared to single lncRNAs with a AUC of 0.83 (95%CI: 0.79-0.86). Fagan's Nomogram ($PLR = 3$) indicated patients with IS are three times more likely to have positive results than health control person (Figure 7b). Only lncRNA-[AC136007.2](#) studied by Li et al. (37) fell on the boundary line of scope with $PLR > 10$ and $NLR < 0.1$ (Figure 7a), whereas the combined lncRNAs were

not in this range. Until now the the number of studies about combined lncRNAs is limited, which maybe contributed to the drawbacks. Hence, more and more larger studies are needed to access the diagnostic accuracy of combined lncRNAs for IS.

To our knowledge, this is the first comprehensive meta-analysis to explore the potential value of ncRNAs in the diagnosis of IS. In our study, we not only used ROC plane, sensitivity analysis, bivariate boxplot, and meta-regression to identify the source of heterogeneity but also performed a goodness-of-fit, bivariate normality, influence analysis, and outlier detection to confirm the results. Moreover, both miRNAs and lncRNAs were included in our study, providing new potential biomarkers for clinical practice. Additionally, we also assessed single and combined ncRNAs to explore the best diagnostic biomarkers for IS.

However, several limitations of this meta-analysis should be emphasized. First, significant heterogeneity existed among the involved studies and in the subgroup analysis. ROC plane were carried out to explore whether heterogeneity caused by threshold effect or not. And the results showed atypical shoulder arm, suggesting nil threshold effect in miRNAs and lncRNAs. Bivariate boxplot analysis revealed that there were respectively 4 studies and 3 studies contributed to the significant heterogeneity in miRNAs and lncRNAs analysis. After excluding these studies, the diagnostic efficiency were remain the same, while heterogeneity was decreased. Additionally, meta-regression analysis of miRNAs indicated that sample size and publish year may also account for high heterogeneity. Other factors, such as the variance of the measuring apparatus and the use of different cut-off values between studies may also be responsible for this heterogeneity. Second, nearly all the studies included in this meta-analysis analyzed data from Asian cohorts, and there is a lack of data regarding ncRNAs and IS in other ethnicities. Third, other sample types, such as cerebrospinal fluid, may also be useful as biomarkers, and should be investigated in future studies. Fourth, as previously mentioned, most studies included in this analysis were retrospective case-control studies, as such they were limited by the different sample sizes and inclusion criteria between studies. Lastly, Deek's funnel plot suggested that publication bias exists for miRNAs. Studies without full texts, such as case reports, conference abstracts, and non-English literature, were excluded from our study, which likely contributed to this publication bias to some degree. Furthermore, negative results are less likely to be published, leading to the exaggeration of results in terms of diagnostic potency. Hence, the results here need to be interpreted with caution.

In conclusion, our study revealed that blood circulating ncRNAs could be a moderately effective candidate biomarker for the diagnosis of IS. Furthermore, while combined lncRNAs showed more accurate diagnostic properties than single lncRNAs, some single miRNAs (e.g. miR-107) showed better diagnostic performance and require more attention. Further research with more extensive statistics should be conducted to confirm our analysis in the future. Studies regarding other types of ncRNAs, such as circular RNAs, are also needed to better understand the diagnostic potential of ncRNAs.

Abbreviations

IS: ischemic stroke;ncRNA: non-coding RNA;ncRNA: long non-coding RNA;miRNA: microRNA;circRNA: circular RNA;SEN: sensitivity ;SPE: specificity;PLR: positive likelihood ratios;NLR: negative likelihood ratios ;TP: true positive;FP: false positive ;FN: false negative ;TN: true negative;DOR: diagnostic odds ratio ;95%CI: 95% confidence intervals;SROC: summary receiver operator characteristic;AUC: area under SROC curve ;MACO: middle cerebral artery occlusion;OGD/R: oxygen and glucose deprivation/reperfusion;qRT-PCR: quantitative real-time PCR;l/R: ischaemia/reperfusion

Declarations

Ethical Statement

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Conflict of interest

The authors have no conflict of interests.

Funding

This study was supported by Natural Science Foundation of Liaoning province of China(2019-MS-364) and the National Natural Science Foundation of China (81501006).

Authors' contributions

Designed the experiments: RZ, JY. Performed the experiments: QW, Analyzed the data:JZ.Wrote the paper: RZ,JY,JZ.All authors read and approved the final manuscript.

Acknowledgments

We are deeply grateful to all participants of this study.

References

1. GBD 2016 Stroke Collaborators. Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* 2019 May;18(5):439-458.
2. Zerna C, Thomalla G, Campbell BCV, Rha JH, Hill MD. Current practice and future directions in the diagnosis and acute treatment of ischaemic stroke. *Lancet.* 2018 Oct 6;392 (10154):1247-1256.
3. Ren X, Hu H, Farooqi I, Simpkins JW. Blood substitution therapy rescues the brain of mice from ischemic damage. *Nat Commun.* 2020 Aug 25;11(1):4078.
4. Kadir RRA, Alwjwaj M, Bayraktutan U. MicroRNA: An Emerging Predictive, Diagnostic, Prognostic and Therapeutic Strategy in Ischaemic Stroke. *Cell Mol Neurobiol.* 2020 Dec 24.
5. Wang SW, Liu Z, Shi ZS. Non-Coding RNA in Acute Ischemic Stroke: Mechanisms, Biomarkers and Therapeutic Targets. *Cell Transplant.* 2018 Dec;27(12):1763-1777.
6. Vasudeva K, Dutta A, Munshi A. Role of lncRNAs in the Development of Ischemic Stroke and Their Therapeutic Potential. *Mol Neurobiol.* 2021 Aug;58(8):3712-3728.
7. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.* 2004 Jan 23;116(2):281-97.
8. Reid G, Kirschner MB, van Zandwijk N. Circulating microRNAs: Association with disease and potential use as biomarkers. *Crit Rev Oncol Hematol.* 2011 Nov;80(2):193-208.
9. Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K, Muniappa N, Tanaka WK, Johnson JM, Sina JF, Fare TL, Sistare FD, Glaab WE. Plasma MicroRNAs as sensitive and specific biomarkers of tissue injury. *Clin Chem.* 2009 Nov;55(11):1977-83.
10. Wang Q, Liu X, Zhu R. Long Noncoding RNAs as Diagnostic and Therapeutic Targets for Ischemic Stroke. *Curr Pharm Des.* 2019;25(10):1115-1121.
11. Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. *Nat Rev Genet.* 2009 Mar;10(3):155-9.
12. Li M, Zhang J. Circulating MicroRNAs: Potential and Emerging Biomarkers for Diagnosis of Cardiovascular and Cerebrovascular Diseases. *Biomed Res Int.* 2015;2015:730535.
13. Ghafouri-Fard S, Shoorei H, Taheri M. Non-coding RNAs participate in the ischemia-reperfusion injury. *Biomed Pharmacother.* 2020 Sep;129:110419.
14. Vemuganti R. All's well that transcribes well: non-coding RNAs and post-stroke brain damage. *Neurochem Int.* 2013 Nov;63(5):438-49.

15. Jeyaseelan K, Lim KY, Armugam A. MicroRNA expression in the blood and brain of rats subjected to transient focal ischemia by middle cerebral artery occlusion. *Stroke*. 2008 Mar;39(3):959-66.
16. Dharap A, Nakka VP, Vemuganti R. Effect of focal ischemia on long noncoding RNAs. *Stroke*. 2012 Oct;43(10):2800-2.
17. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM; QUADAS-2 Group. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med*. 2011 Oct 18;155(8):529-36.
18. Cheng X, Kan P, Ma Z, Wang Y, Song W, Huang C, Zhang B. Exploring the potential value of miR-148b-3p, miR-151b and miR-27b-3p as biomarkers in acute ischemic stroke. *Biosci Rep*. 2018 Nov 28;38(6):BSR20181033.
19. Gao J, Yang S, Wang K, Zhong Q, Ma A, Pan X. Plasma miR-126 and miR-143 as Potential Novel Biomarkers for Cerebral Atherosclerosis. *J Stroke Cerebrovasc Dis*. 2019 Jan;28(1):38-43.
20. Jin F, Xing J. Circulating pro-angiogenic and anti-angiogenic microRNA expressions in patients with acute ischemic stroke and their association with disease severity. *Neurol Sci*. 2017 Nov;38(11):2015-2023.
21. Jin F, Xing J. Circulating miR-126 and miR-130a levels correlate with lower disease risk, disease severity, and reduced inflammatory cytokine levels in acute ischemic stroke patients. *Neurol Sci*. 2018 Oct;39(10):1757-1765.
22. Kim JM, Jung KH, Chu K, Lee ST, Ban J, Moon J, Kim M, Lee SK, Roh JK. Atherosclerosis-Related Circulating MicroRNAs as a Predictor of Stroke Recurrence. *Transl Stroke Res*. 2015 Jun;6(3):191-7.
23. Liu G, Cao C, Zhu M. Peripheral Blood miR-451 May Serve as a Biomarker of Ischemic Stroke. *Clin Lab*. 2019 Sep 1;65(9).
24. Long G, Wang F, Li H, Yin Z, Sandip C, Lou Y, Wang Y, Chen C, Wang DW. Circulating miR-30a, miR-126 and let-7b as biomarker for ischemic stroke in humans. *BMC Neurol*. 2013 Nov 16;13:178.
25. Lu WJ, Zeng LL, Wang Y, Zhang Y, Liang HB, Tu XQ, He JR, Yang GY. Blood microRNA-15a Correlates with IL-6, IGF-1 and Acute Cerebral Ischemia. *Curr Neurovasc Res*. 2018;15(1):63-71.
26. Peng G, Yuan Y, Wu S, He F, Hu Y, Luo B. MicroRNA let-7e Is a Potential Circulating Biomarker of Acute Stage Ischemic Stroke. *Transl Stroke Res*. 2015 Dec;6(6):437-45.
27. Tian C, Li Z, Yang Z, Huang Q, Liu J, Hong B. Plasma MicroRNA-16 Is a Biomarker for Diagnosis, Stratification, and Prognosis of Hyperacute Cerebral Infarction. *PLoS One*. 2016;11(11):e0166688.
28. Tiedt S, Prestel M, Malik R, Schieferdecker N, Duering M, Kautzky V, Stoycheva I, Böck J, Northoff BH, Klein M, Dorn F, Krohn K, Teupser D, Liesz A, Plesnila N, Holdt LM, Dichgans M. RNA-Seq Identifies

- Circulating miR-125a-5p, miR-125b-5p, and miR-143-3p as Potential Biomarkers for Acute Ischemic Stroke. *Circ Res*. 2017 Sep 29;121(8):970-980.
29. Yang ZB, Li TB, Zhang Z, Ren KD, Zheng ZF, Peng J, Luo XJ. The Diagnostic Value of Circulating Brain-specific MicroRNAs for Ischemic Stroke. *Intern Med*. 2016;55(10):1279-86.
30. Zeng L, Liu J, Wang Y, Wang L, Weng S, Tang Y, Zheng C, Cheng Q, Chen S, Yang GY. MicroRNA-210 as a novel blood biomarker in acute cerebral ischemia. *Front Biosci (Elite Ed)*. 2011 Jun 1;3:1265-72.
31. Zhao B, Zhu Z, Hao J, Wan Z, Guo X. Decreased plasma miR-335 expression in patients with acute ischemic stroke and its association with calmodulin expression. *J Int Med Res*. 2016 Dec;44(6):1331-1338.
32. Zhou J, Chen L, Chen B, Huang S, Zeng C, Wu H, Chen C, Long F. Increased serum exosomal miR-134 expression in the acute ischemic stroke patients. *BMC Neurol*. 2018 Dec 4;18(1):198.
33. Deng QW, Li S, Wang H, Sun HL, Zuo L, Gu ZT, Lu G, Sun CZ, Zhang HQ, Yan FL. Differential long noncoding RNA expressions in peripheral blood mononuclear cells for detection of acute ischemic stroke. *Clin Sci (Lond)*. 2018 Jul 31;132(14):1597-1614.
34. Feng L, Guo J, Ai F. Circulating long noncoding RNA ANRIL downregulation correlates with increased risk, higher disease severity and elevated pro-inflammatory cytokines in patients with acute ischemic stroke. *J Clin Lab Anal*. 2019 Jan;33(1):e22629.
35. Guo X, Yang J, Liang B, Shen T, Yan Y, Huang S, Zhou J, Huang J, Gu L, Su L. Identification of Novel LncRNA Biomarkers and Construction of LncRNA-Related Networks in Han Chinese Patients with Ischemic Stroke. *Cell Physiol Biochem*. 2018;50(6):2157-2175.
36. Li P, Duan S, Fu A. Long noncoding RNA NEAT1 correlates with higher disease risk, worse disease condition, decreased miR-124 and miR-125a and predicts poor recurrence-free survival of acute ischemic stroke. *J Clin Lab Anal*. 2020 Feb;34(2):e23056.
37. Li S, Zheng H, Chen L, Xu C, Qu X, Qin Z, Gao J, Li J, Liu J. Expression Profile and Potential Functions of Circulating Long Noncoding RNAs in Acute Ischemic Stroke in the Southern Chinese Han Population. *Front Mol Neurosci*. 2019 Nov 29;12:290.
38. Wang J, Zhao H, Fan Z, Li G, Ma Q, Tao Z, Wang R, Feng J, Luo Y. Long Noncoding RNA H19 Promotes Neuroinflammation in Ischemic Stroke by Driving Histone Deacetylase 1-Dependent M1 Microglial Polarization. *Stroke*. 2017 Aug;48(8):2211-2221.
39. Wang J, Ruan J, Zhu M, Yang J, Du S, Xu P, Zhang Z, Wang P, Yang W, Yu M. Predictive value of long noncoding RNA ZFAS1 in patients with ischemic stroke. *Clin Exp Hypertens*. 2019;41(7):615-621.

40. Zhu M, Li N, Luo P, Jing W, Wen X, Liang C, Tu J. Peripheral Blood Leukocyte Expression of lncRNA MIAT and Its Diagnostic and Prognostic Value in Ischemic Stroke. *J Stroke Cerebrovasc Dis.* 2018 Feb;27(2):326-337.
41. Koutsis G, Siasos G, Spengos K. The emerging role of microRNA in stroke. *Curr Top Med Chem.* 2013;13(13):1573-88.
42. Wang W, Sun G, Zhang L, Shi L, Zeng Y. Circulating microRNAs as novel potential biomarkers for early diagnosis of acute stroke in humans. *J Stroke Cerebrovasc Dis.* 2014 Nov-Dec;23(10):2607-2613.
43. Sepramaniam S, Tan JR, Tan KS, DeSilva DA, Tavintharan S, Woon FP, Wang CW, Yong FL, Karolina DS, Kaur P, Liu FJ, Lim KY, Armugam A, Jeyaseelan K. Circulating microRNAs as biomarkers of acute stroke. *Int J Mol Sci.* 2014 Jan 20;15(1):1418-32.
44. Li P, Teng F, Gao F, Zhang M, Wu J, Zhang C. Identification of circulating microRNAs as potential biomarkers for detecting acute ischemic stroke. *Cell Mol Neurobiol.* 2015 Apr;35(3):433-447.
45. Dewdney B, Trollope A, Moxon J, Thomas Manapurathe D, Biros E, Golledge J. Circulating MicroRNAs as Biomarkers for Acute Ischemic Stroke: A Systematic Review. *J Stroke Cerebrovasc Dis.* 2018 Mar;27(3):522-530.
46. Eyileten C, Wicik Z, De Rosa S, Mirowska-Guzel D, Soplinska A, Indolfi C, Jastrzebska-Kurkowska I, Czlonkowska A, Postula M. MicroRNAs as Diagnostic and Prognostic Biomarkers in Ischemic Stroke-A Comprehensive Review and Bioinformatic Analysis. *Cells.* 2018 Dec 6;7(12):249.
47. Dykstra-Aiello C, Jickling GC, Ander BP, Shroff N, Zhan X, Liu D, Hull H, Orantia M, Stamova BS, Sharp FR. Altered Expression of Long Noncoding RNAs in Blood After Ischemic Stroke and Proximity to Putative Stroke Risk Loci. *Stroke.* 2016 Dec;47(12):2896-2903.
48. He W, Wei D, Cai D, Chen S, Li S, Chen W. Altered Long Non-Coding RNA Transcriptomic Profiles in Ischemic Stroke. *Hum Gene Ther.* 2018 Jun;29(6):719-732.
49. Bao MH, Szeto V, Yang BB, Zhu SZ, Sun HS, Feng ZP. Long non-coding RNAs in ischemic stroke. *Cell Death Dis.* 2018 Feb 15;9(3):281.
50. Akella A, Bhattarai S, Dharap A. Long Noncoding RNAs in the Pathophysiology of Ischemic Stroke. *Neuromolecular Med.* 2019 Dec;21(4):474-483.
51. Zhou Q, Liu J, Quan J, Liu W, Tan H, Li W. MicroRNAs as potential biomarkers for the diagnosis of glioma: A systematic review and meta-analysis. *Cancer Sci.* 2018;109(9):2651-2659. doi:10.1111/cas.13714
52. Yang ZB, Zhang Z, Li TB, Lou Z, Li SY, Yang H, Yang J, Luo XJ, Peng J. Up-regulation of brain-enriched miR-107 promotes excitatory neurotoxicity through down-regulation of glutamate transporter-1

53. Cheng H, Sun M, Wang ZL, Wu Q, Yao J, Ren G, Sun XL. LncRNA RMST-mediated miR-107 transcription promotes OGD-induced neuronal apoptosis via interacting with hnRNPK. *Neurochem Int.* 2020 Feb;133:104644.

Table 1 Summary of single and combined miRNAs from retrieved 15 articles

	Author year	ethnicity	method	miRNA	TP	FP	FN	TN
1	Cheng 2018	Asian	qRT-PCR	miR-148b-3p	40	8	37	34
				miR-151b	33	3	44	39
				miR-27b-3p	39	9	38	33
				miR-148b and miR-151b	51	3	26	39
				miR-148b-3p and miR-27b-3p	52	3	25	39
				miR-151b and miR-27b-3p	21	3	56	39
				miR-148b, miR-151b and miR-27b-3p	40	3	37	39
2	Gao 2019	Asian	qRT-PCR	miR-126	84	13	14	37
				miR-143	36	16	62	34
3	Jin 2017	Asian	qRT-PCR	miR-126, miR-130a, miR-222, miR-218, and miR-185	93	50	13	60
4	Jin 2018	Asian	qRT-PCR	miR-126, miR-130a, miR-222, miR-218, and miR-185	124	45	24	103
5	Kim 2015	Asian	qRT-PCR	miRNA-17	31	4	52	33
6	Liu 2019	Asian	qRT-PCR	miR-451	271	10	31	292
7	Long 2013	Asian	qRT-PCR	let-7b	35	8	3	42
				miR-126	35	8	3	42
				miR-30a	36	10	2	40
8	Lu 2018	Asian	qRT-PCR	miR-15a	22	1	57	74
9	Peng 2015	Asian	qRT-PCR	let-7e	60	14	12	37
			qRT-PCR	miR-338	52	24	20	27
10	Tian 2016	Asian	qRT-PCR	miR-16	28	4	12	26
11	Tiedt 2017	Europe	qRT-PCR	miR-125a-5p, miR-125b-5p and miR-143-3p	171	24	29	76
12	Yang 2016	Asian	qRT-PCR	miR-107	107	5	7	53
				miR-128b	83	5	31	53

				miR-153	104	15	10	43
13	Zeng 2011	Asian	qRT-PCR	miRNA-210	99	35	13	25
14	Zhao 2016	Asian	qRT-PCR	miR-335	164	32	4	72
15	Zhou 2018	Asian	qRT-PCR	miR-134	38	14	12	36

Table 2 Summary of single and combined lncRNAs from retrieved 8 studies

	Author year	ethnicity	method	lncRNA	TP	FP	FN	TN
1	Deng 2018	Asian	qRT-PCR	linc-DHFRL1-4	22	9	10	23
				SNHG15	19	5	13	27
				linc-FAM98A-3	19	10	13	22
				linc-DHFRL1-4, SNHG15, linc-FAM98A-3	26	9	6	23
2	Feng 2019	Asian	qRT-PCR	lncRNA ANRIL	91	36	35	89
3	Guo 2018	Asian	qRT-PCR	ENST00000568297	32	18	18	32
				ENST00000568243	35	15	15	35
				NR_046084	31	15	19	35
				ENST00000568297, ENST00000568243	38	12	12	38
				ENST00000568297,NR_046084	35	16	15	34
				ENST00000568243,NR_046084	39	12	11	38
				ENST00000568297, ENST00000568243, and NR_046084	41	10	9	40
4	Li 2020	Asian	qRT-PCR	lncRNA NEAT1	135	36	75	174
5	Li 2019	Asian	qRT-PCR	lncRNA-C14orf64	20	8	12	24
				lncRNA-AC136007.2	29	3	3	29
6	Wang 2017	Asian	qRT-PCR	H19	29	2	7	23
7	Wang 2019	Asian	qRT-PCR	ZFAS1	59	57	7	54
8	Zhu 2018	Asian	qRT-PCR	MIAT	140	37	49	152

Figures

Figure 1

Flowchart of article selection

Figure 2

Forest plots of miRNAs in diagnosing IS from HCs, including sensitivity and specificity

Figure 3

SROC curve for 27 sets of miRNAs in 15 studies,

Figure 4

Assessment of the clinical applicability of microRNAs (miRNAs) for diagnosis. (a) Summary of PLR and NLR for diagnosis of IS. (b) Fagan’s nomogram of the miRNA tests for diagnosis of IS

Figure 5

Forest plots of lncRNAs in diagnosing IS from HCs, including sensitivity and specificity

Figure 6

SROC curve for 18 sets of lncRNAs in 8 studies

Figure 7

Assessment of the clinical applicability of lncRNAs for diagnosis. (a) Summary of PLR and NLR for diagnosis of IS. (b) Fagan's nomogram of the lncRNA tests for diagnosis of IS

Figure 8

Diagrams of miRNAs: (a) ROC plane, (b) Meta-regression analysis, (c) Influence analysis, (d) Bivariate boxplot

Figure 9

Deek's funnel plot of miRNAs

Figure 10

Diagrams of lncRNAs: (a) ROC plane, (d) Meta-regression analysis, (b) Influence analysis, (c) Bivariate boxplot

Figure 11

Deek's funnel plot of lncRNAs

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterials.docx](#)