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

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Received: 11 January 2022 / Accepted: 25 February 2022 / Published online: 5 April 2022
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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 to clinical practice. Therefore, we performed a 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 differentially expressed ncRNAs and IS. Pooled sensitivity and specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), and the corresponding 95% confidence intervals (95% CIs) were calculated to assess the diagnostic performance of ncRNAs.

Results Fifteen studies on microRNAs (miRNAs) including 1687 IS patients and eight studies on long non-coding RNAs (lncRNAs) including 741 IS patients were included in our study. Estimates of the identification efficiency of miRNAs from the 15 studies were as follows: 0.76 (95% CI: 0.67–0.84) for sensitivity (SEN), 0.83 (95% CI: 0.77–0.88) for specificity (SPE), 4.5 (95% CI: 3.4–6.1) for PLR, 0.29 (95% CI: 0.20–0.40) for NLR, and 16 (95% CI: 10–26) for DOR. The area under the curve (AUC) of the summary receiver operator characteristic (SROC) curve showed diagnostic accuracy of 0.87 (95% CI: 0.84–0.90); thus the diagnostic value of all the miRNAs was moderate. In addition, the results for lncRNAs were as follows: 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, and 8 (95% CI: 6–11) for DOR, 0.80 (95% CI: 0.76–0.83) for AUC, and 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, the 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.

Keywords Ischemic stroke · miRNAs · lncRNAs · Diagnosis · Biomarker

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 GBD (2016). 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 (Zerna et al. 2018; Ren et al. 2020). 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 (Kadir et al. 2020; Wang et al. 2018; Vasudeva et al. 2021).

It was recently demonstrated that ncRNAs are involved in the pathogenesis of IS, including neuroinflammation,
apoptosis, atherosclerosis, and angiogenesis (Wang et al. 2018; Vasudeva et al. 2021). ncRNAs are defined as non-protein-coding transcripts and are classified into many types according to their length and connection, including microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs). lncRNAs are a type of linear RNA over 200 nucleotides in length, whereas miRNAs are 22-nucleotide-long ncRNAs (Bartel 2004). 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 (Reid et al. 2011; Laterza et al. 2009; Wang et al. 2019a). MiRNAs regulate gene expression at the post-transcriptional level by binding to the 3′ untranslated region of mRNA, whereas lncRNAs exert their effects at the transcriptional and post-transcriptional levels (Bartel 2004; Mercer et al. 2009). Recently, emerging studies have begun to reveal that ncRNAs can accumulate and be expressed in the mammalian brain and in neuronal cell lines and play several roles in physiological and pathological activities in the brain (Li and Zhang et al. 2015).

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 (Ghafouri-Fard et al. 2020; Vemuganti 2013; Jeyaseelan et al. 2008; Dharap et al. 2012). However, discrepant reports make it difficult to apply ncRNAs to clinical practice. Moreover, not all ncRNAs are suitable 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.

Materials and Methods

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 consensus was reached.

Inclusion Criteria

Eligible studies met the following criteria: (1) studies were focused on the diagnostic performance of lncRNAs or miRNAs in IS and (2) described peripheral blood detection (plasma, serum, and whole blood); (3) all patients were diagnosed with IS according to radiological imaging (CT or MRI) and neurological examination; and (4) sufficient data were available, including the sample size of cases and controls, sensitivity (SEN), specificity (SPE), and area under the curve (AUC). In addition, the exclusion criteria were as follows: (1) literature not in English; (2) reviews, meeting records, or letters; (3) studies not conducted in humans; or (4) not describing peripheral blood detection of lncRNAs and miRNAs.

Data Extraction and Quality Assessment

Two researchers extracted information from included studies, as follows: 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) (Whiting et al. 2020), which contain four domains: patient selection, index test, reference standard, and flow and timing. Each domain is used for bias assessment and the first three domains were applied to evaluate clinic applicability of these studies.

Statistical Analysis

The extracted data were analyzed using STATA 15.0 and p < 0.05 was considered statistically significant. Pooled SEN and 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 and the Fagan 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 analyses including goodness-of-fit, bivariate normality, influence analysis, and outlier
detection were used to validate our results. A funnel plot was used to examine publication bias.

**Results**

**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 the following: 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 (Cheng et al. 2018; Gao et al. 2019; Jin and Xing 2017, 2018; Kim et al. 2015; Liu et al. 2019; Long et al. 2013; Lu et al. 2018; Peng et al. 2015; Tian et al. 2016; Tiedt et al. 2017; Yang et al. 2016; Zeng et al. 2011; Zhao et al. 2016; Zhou et al. 2018). A flowchart of the whole selection process is shown in Fig. 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 quintuple) were described in terms of their diagnostic value for IS in 20 and seven (three sets of double, two sets of triple, and two sets of quintuple miRNAs) studies, respectively (Table 1). Meanwhile, eight studies were conducted for IncRNAs, encompassing 741 qualifying patients and 774 corresponding controls. There were 18 sets of IncRNAs performed in total, from which single and combined IncRNAs (double and triple) were described in 13 and five (three sets of double and two sets of triple IncRNAs) studies, respectively (Table 2).

**Diagnostic Value of NcRNAs**

**Diagnostic Value of miRNA**

The specificity and sensitivity of miRNAs in the diagnosis of IS are shown in forest plots for the 27 sets of miRNAs from the 15 selected studies (Fig. 2). These plots indicate significant heterogeneity, with $I^2$ values of 95.74 and 89.43 for sensitivity and specificity, respectively; therefore, 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,
Table 1  Summary of single and combined miRNAs from 15 retrieved articles

| Author year          | Ethnicity | Method   | miRNA                          | TP | FP | FN | TN |
|----------------------|-----------|----------|--------------------------------|----|----|----|----|
| Cheng et al. (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 |
| Gao et al. (2019)    | Asian     | qRT-PCR  | miR-126                        | 84 | 13 | 14 | 37 |
|                      |           |          | miR-143                        | 36 | 16 | 62 | 34 |
| Jin and Xing (2017)  | Asian     | qRT-PCR  | miR-126, miR-130a, miR-222, miR-218, and miR-185 | 93 | 50 | 13 | 60 |
| Jin and Xing (2018)  | Asian     | qRT-PCR  | miR-126, miR-130a, miR-222, miR-218, and miR-185 | 124 | 45 | 24 | 103 |
| Kim et al. (2015)    | Asian     | qRT-PCR  | miRNA-17                       | 31 | 4  | 52 | 33 |
| Liu et al. (2019)    | Asian     | qRT-PCR  | miR-451                        | 271 | 10 | 31 | 292 |
| Long et al. (2013)   | Asian     | qRT-PCR  | let-7b                         | 35 | 8  | 3  | 42 |
|                      |           |          | miR-126                        | 35 | 8  | 3  | 42 |
|                      |           |          | miR-30a                        | 36 | 10 | 2  | 40 |
| Lu et al. (2018)     | Asian     | qRT-PCR  | miR-15u                        | 22 | 1  | 57 | 74 |
| Peng et al. (2015)   | Asian     | qRT-PCR  | let-7e                         | 60 | 14 | 12 | 37 |
|                      |           |          | miR-338                        | 52 | 24 | 20 | 27 |
| Tian et al. (2016)   | Asian     | qRT-PCR  | miR-16                         | 28 | 4  | 12 | 26 |
| Tiedt et al. (2017)  | European  | qRT-PCR  | miR-125a-5p, miR-125b-5p and miR-143-3p | 171 | 24 | 29 | 76 |
| Yang et al. (2016)   | Asian     | qRT-PCR  | miR-107                        | 107 | 5  | 7  | 53 |
|                      |           |          | miR-128b                       | 83 | 5  | 31 | 53 |
|                      |           |          | miR-153                        | 104 | 15 | 10 | 43 |
| Zeng et al. (2011)   | Asian     | qRT-PCR  | miRNA-210                      | 99 | 35 | 13 | 25 |
| Zhao et al. (2016)   | Asian     | qRT-PCR  | miR-335                        | 164 | 32 | 4  | 72 |
| Zhou et al. (2018)   | Asian     | qRT-PCR  | miR-134                        | 38 | 14 | 12 | 36 |

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

| Author year          | Ethnicity | Method   | lncRNA                          | TP | FP | FN | TN |
|----------------------|-----------|----------|--------------------------------|----|----|----|----|
| Deng et al. (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 |
| Feng et al. (2019)   | Asian     | qRT-PCR  | lncRNA ANRIL                    | 91 | 36 | 35 | 89 |
| Guo et al. (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 |
| Li et al. (2020)     | Asian     | qRT-PCR  | lncRNA NEAT1                    | 135 | 36 | 75 | 174 |
| Li et al. (2019)     | Asian     | qRT-PCR  | lncRNA-C14orf64                 | 20  | 8  | 12 | 24 |
|                      |           |          | lncRNA-AC136007.2               | 29  | 3  | 3  | 29 |
| Wang et al. (2017)   | Asian     | qRT-PCR  | H19                             | 29  | 2  | 7  | 23 |
| Wang et al. (2019)   | Asian     | qRT-PCR  | ZFAS1                           | 59  | 57 | 7  | 54 |
| Zhu et al. (2018)    | Asian     | qRT-PCR  | MIAT                            | 140 | 37 | 49 | 152 |
0.29 (95% CI: 0.20–0.40) for NLR, and 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 (Fig. 3). In subgroup analysis based on the 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, and 14 (95% CI: 9–21) and 17 (95% CI: 9–32) for DOR, respectively. 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 (Figs. S1 and S2 in supplementary materials). In summary for the PLR and NLR, the 22nd set of miRNA (miR-107) from the study by Yang et al. (2016) had a PLR > 10 and NLR < 0.1 (Fig. 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 pre-test probability of 20% (Fig. 4b).

**Diagnostic Value of lncRNA**

Statistics regarding lncRNAs extracted from eight selected studies were analyzed in the same way. As shown in the forest plots (Fig. 5), significant heterogeneity was found, with $I^2$ values of 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, and 0.36 (95% CI: 0.31–0.42) for AUC (Fig. 6). In subgroup analysis based on the lncRNA profile, estimates of combined lncRNAs and single lncRNAs were as follows: 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, and 10 (95% CI: 6–15) and 7 (95% CI: 5–11) for DOR. Compared with single lncRNAs, combined lncRNAs had higher diagnostic accuracy, with an AUC of 0.83 (95% CI: 0.79–0.86) versus 0.79 (95% CI: 0.75–0.82), as shown in Figs. S3 and S4 (supplementary materials). In summary for the PLR and NLR, no study had a PLR $>$ 10 and NLR $<$ 0.1, and only the 15th set of lncRNAs (lncRNA-AC136007.2) conducted by Li et al. (2019) fell on the borderline (Fig. 7a). In the Fagan nomogram, when the pre-test 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 for lncRNAs was 42% and 8%, respectively (Fig. 7b).

**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, 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 (Fig. 8a). Influence analysis showed that the study conducted by Liu et al. (2019) may have had an influence on heterogeneity (Fig. 8c). The bivariate boxplot revealed that four studies (Liu et al. 2019; Lu et al. 2018; Peng et al. 2015; Zeng et al. 2011) contributed to this high heterogeneity (Fig. 8d). After excluding these four studies, the pooled estimates did not change, whereas the heterogeneity in sensitivity and specificity decreased (Fig. S5). To confirm this result, we conducted further analysis for heterogeneity using goodness-of-fit, bivariate normality, influence analysis,
and outlier detection, and found similar results (Fig. S6). We read these four 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). We found that sample size and publication year may have accounted for part of the 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 (Fig. 8b). Publication bias existed across the included studies on miRNAs, as identified by the Deeks funnel plot ($p < 0.05$) (Fig. 9).

As for lncRNAs, significant heterogeneity existed among the eight lncRNA studies in the forest plots (SEN: $I^2 = 56.69$, SPE: $I^2 = 74.97$) (Fig. 5). Similarly, ROC plane, sensitivity, bivariate boxplot, and meta-regression analyses were performed. The ROC plane showed that significant heterogeneity did not result from the threshold effect (Fig. 10a). The influence analysis showed that no study exerted a substantial influence on the results (Fig. 10c). Three studies (Li 2019; Wang 2017, 2019b) were excluded from the boxplot and...
may have been the source of heterogeneity (Fig. 10d). After excluding these three studies, the pooled estimates were similar to the previous results, whereas the heterogeneity in sensitivity and specificity decreased (SEN: $I^2 = 27.71$, SPE: $I^2 = 29.26$) (Fig. S7). Subsequently, further analysis including goodness-of-fit, bivariate normality, influence analysis, and outlier detection for heterogeneity showed the same result (Fig. S8). Another possible source of heterogeneity indicated by meta-regression analysis was sample size (> 50 or not), with $p < 0.01$ for sensitivity and $p < 0.001$ for specificity (Fig. 10b). No significant publication bias was found in Deeks’ funnel plot ($p = 0.91$; Fig. 11).

Discussion

Several studies have reported aberrant expression of miRNAs and lncRNAs in patients with IS (Koutsis et al. 2013; Wang et al. 2014; Sepramaniam et al. 2014; Li et al. 2015; Dewdney et al. 2018; Eyileten et al. 2018; Dykstra-Aiello et al. 2016; He et al. 2018; Bao et al. 2018; Akella et al. 2019). However, the results of these studies have been inconsistent and have not yet reached a consensus. Some are significantly upregulated, while others are downregulated in the circulating blood of patients with IS. 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 systematic meta-analysis to clarify confusion about the diagnostic value of circulating ncRNAs, which would provide suggestions for clinical applicability.

Our meta-analysis revealed 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 (Figs. S1 and S2 in supplementary materials). Clinical applicability for diagnosis is dependent on the summary of PLR, NLR, and Fagan’s nomogram. In summary for PLR and NLR, PLR > 10 and NLR < 0.1 represent higher diagnostic accuracy (Zhou et al. 2018). Among the miRNAs, miR-107 as reported by Yang et al. (2016) was within this range (Fig. 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 levels were higher in IS patients than those in healthy controls, but miR-107 showed the greatest diagnostic value, with higher specificity and sensitivity. Yang et al. (2014) found that miRNA-107 promoted excitatory neurotoxicity by regulating glutamate transporter-1 expression in a rat model of ischemia–reperfusion injury. Additionally, Cheng et al. (2020) found that miRNA-107 participated in neuronal apoptosis via the p53 pathway in
OGD-treated HT-22 hippocampal neuron cells. A recent study by Li et al. demonstrated that miRNA-107 was involved in angiogenesis after stroke by targeting Dicer-1 (Dewdney et al. 2018). In conclusion, miR-107 plays an important role in stroke pathophysiology. Hence, miR-107 represents a novel candidate biomarker for differentiation of patients with IS from healthy individuals, and requires further intensive study. Additionally, the DOR of miRNAs was 16, confirming that miRNAs are promising diagnostic tools for IS.

Multiple studies have reported that single lncRNAs exhibit good diagnostic performance for clinical use (Feng et al. 2019, Li et al. 2019, 2020; Wang et al. 2017, 2019b; Zhu et al. 2018). However, Deng et al. (2018) 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 controls. In our analysis, we summarized lncRNAs associated with IS, and found that combined lncRNAs yielded better diagnostic capability than single lncRNAs, with an AUC of 0.83 (95% CI: 0.79–0.86). Fagan’s nomogram (PLR = 3) indicated that patients with IS were three times as likely as healthy controls to have positive results (Fig. 7b). Only lncRNA-AC136007.2, studied by Li et al. (2019), fell on the boundary line of the range with PLR > 10 and NLR < 0.1 (Fig. 7a), whereas the combined lncRNAs were not in this range. Until now, the number of studies on combined lncRNAs has been limited, which may have contributed to these drawbacks. Hence, more studies are needed to assess the diagnostic accuracy of combined lncRNAs in 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, in addition to using ROC plane, sensitivity analysis, bivariate boxplot, and meta-regression to identify the source of heterogeneity, we also performed 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. 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 was used to explore whether the heterogeneity was caused by the threshold effect. The results showed an atypical shoulder arm, suggesting no threshold effect in miRNAs and lncRNAs. Bivariate boxplot analysis revealed that four and three studies contributed to the significant heterogeneity in miRNA and lncRNA analyses, respectively. After excluding these studies, the diagnostic efficiency remained the same, whereas heterogeneity decreased. Additionally, meta-regression analysis of miRNAs indicated that sample size and publication year could also have accounted for the high heterogeneity. Other factors, such as differences in the measuring apparatus and the use of different cutoff values between studies, may be responsible for this heterogeneity as well. 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, and as such they were limited by the different sample sizes and inclusion criteria between studies. Lastly, Deeks’ 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 IncRNAs showed more accurate diagnostic properties than single IncRNAs, some single miRNAs (e.g., miR-107) showed better diagnostic performance and thus warrant further attention. Additional studies with more extensive statistics are needed to confirm our analysis. Studies regarding other types of ncRNAs, such as circular RNAs, are also needed to better understand the diagnostic potential of ncRNAs.
Fig. 11 Deek’s funnel plot of lncRNAs

Abbreviations IS: Ischemic stroke; ncRNA: Non-coding RNA; lncRNA: 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% CIs: 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; I/R: Ischemia/reperfusion

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12031-022-01991-2.

Acknowledgements We are deeply grateful to all participants of this study.

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.

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).

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

Declarations

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Conflict of Interest The authors have no conflict of interest.

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