

Original Article

Predictive value of microRNAs as novel biomarkers in detection of lymphoma

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Abstract: MicroRNAs (miRNAs) have attracted many attentions in lymphoma diagnostic research. The inconsistency of diagnostic performance in these existed literatures leading us to conduct this meta-analysis. In order to have a scientific and reliable study, all related articles were screened from Medline, Embase, CNKI and other databases. The sensitivity and specificity of each involved research were used to plot the summary receiver operator characteristic (SROC) curve and calculate the area under the curve (AUC). The QUADAS-2 tool was applied to estimate the quality of included studies. In addition, Deeks' funnel plot asymmetry test was performed to estimate publication bias. Overall, 14 studies from 6 articles were included to evaluate the whole test performance. The overall pooled results were as follows: sensitivity was 0.91 (95% CI: 0.83-0.95), specificity was 0.84 (95% CI: 0.75-0.90), the AUC was 0.93 (95% CI: 0.91-0.95), positive likelihood ratio-PLR was 5.5 (95% CI: 3.5-8.8), negative likelihood ratio-NLR was 0.11 (95% CI: 0.06-0.21), and diagnostic odds ratio-DOR was 50 (95% CI: 19-128). In summary, results from meta-analysis showed that miRNAs analysis might significantly increase the diagnostic accuracy of lymphoma. Further massive prospective studies still needed to validate our conclusion before clinical application.

Keywords: Lymphoma, microRNAs, biomarker, diagnostic, meta-analysis

Introduction

Lymphoma, a part of the multitude group of tumors, is a cluster of blood cell tumors that grow from lymphocytes. The clinical feature of lymphoma covers enlarged lymph nodes, fevers, sweats at night and weight loss [1]. Lymphoma makes up 3-4% of all cancers and it is more likely to afflict younger men [2]. According to the pathological diagnosis, it can be divided into two main types: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL). NHL accounts for about 90% of clinical cases and contains several sub-types (diffuse large B cell lymphoma-DLBCL, Burkitt lymphoma, follicular lymphoma-FL, mantle cell lymphoma, and marginal zone lymphoma) [3, 4]. There are many threats for NHL and HL, such as HIV/AIDS, autoimmune diseases, infection with human T-lymphotropic virus and Epstein-Barr virus [5]. Nearly one million people were suffered from lymphomas and around sixty percent deaths in 2012. Up to now, lymphoma has become the seventh-largest cancer in humans

[6, 7], especially in children [8]. Due to the shortage of complete cure and effective diagnostic markers, the two year and five year survival rate of high risk lymphoma patients were only 34% and 26%, respectively. However, the corresponding rates were 84% and 73% in low risk lymphoma patients [9, 10]. Thus, it is urgent to find a suitable biomarker for early diagnosing lymphoma.

MicroRNAs (miRNAs) are endogenous, non-coding, single-strand RNA molecules with a length of about 22-nucleotides (nt) [11]. They have post-transcriptional regulate of the expression of various target genes. Previous researches have confirmed that miRNAs play an important role in regulating cell proliferation, development, differentiation, apoptosis, metabolism, signal transduction and oncogenesis [12]. Researches also confirmed that miRNAs could regulate the expression of oncogenes or anti-oncogenes, which might mean that they take part in the tumorigenesis and progression of lymphoma.

Guo et al. observed plasma miR-221 may be used as a therapeutic goal for treatment of NK/T-cell lymphoma and served as a diagnostic marker with a moderate sensitivity (57.5%) and specificity (75.7%) [13]. Lawrie et al. identified important diagnostic relevance of miRNA level between lymphoma patients and normal controls, which indicated that miR-125b and miR-155 were able to predict the malignant nature of the samples with a success rate of 99% in DLBCL [14]. Another study by Zhong et al. also confirmed the findings of previous works by discovering miR-155 might be useful diagnostically for DLBCL [15]. Furthermore, Fang et al. also reported miR-15a, miR-16-1, miR-34a and miR-155 might be potential mark tools in the DLBCL diagnosis [16]. Another interesting finding from Baraniskin et al. research was one particular miRNA obtained from cerebrospinal fluid (CSF) can serve as a novel biomarker for primary central nervous system lymphoma (PCNSL) diagnosis, therapy and management [17]. Their further results demonstrated that the combined analysis of serum miRNAs (miR-21, -19b, and -92) could diagnoses PCNSL patients with a high sensitivity (97.4%) and specificity (97.4%) [18].

From previous works, we find a variety of miRNAs might be used as potential biomarkers for lymphoma early detection. Different evidences from previous studies could be helpful for us to understand the overall value of miRNAs diagnosis. Therefore, we conducted this meta-analysis of the previous literatures to illustrate the underlying value of miRNAs as biomarkers for the lymphoma diagnosis.

Materials and methods

Literature search

This meta-analysis was administrated according to guidebooks of diagnostic meta-analysis [19, 20]. The Medline, Embase, and CNKI databases were searched on Jan. 2, 2015, with the key words of lymphoma, primary central nervous system lymphoma, non-Hodgkin lymphoma, Hodgkin lymphoma, diffuse large B cell lymphoma; microRNAs, miRNAs, miRs; diagnosis, sensitivity, specificity, ROC curve. We manually searched the reference of relevant reviews to obtain additional articles as well.

Inclusion and exclusion criteria

Eligible studies had to comply with the following inclusion criteria: concern miRNAs as the potential markers for lymphoma diagnostic; provide a standard reference of lymphoma diagnosis; and sufficient data were provided to build two-by-two tables, including true negative-TN and false negative-FN, true positive-TP, false positive FP. Exclusion criteria: studies were irrelevant to the diagnostic values of miRNAs for lymphoma; case reports, editorials, letters or reviews; studies with duplicate data covered in other studies or without qualified data.

Data extraction and quality assessment

Two reviewers independently extracted the following data from all included articles: author, year; country, ethnicity of patients, cases and controls' number, mean age and male ratio, patient spectrum, source of control, detecting method, miRNA profiling, specimen, diagnostic power, sensitivity, and specificity data. The qualities of included studies were measured by Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) with four key domains and seven questions [21]. This is an evidence-based approach which could be used to evaluate diagnostic accuracy of studies.

Statistical analysis

The bivariate meta-analysis model was applied to sum the sensitivity, specificity, PLR, NLR, and DOR [22]. The summary receiver operator characteristic (SROC) curve and the area under the curve (AUC) were derived from the sensitivity and specificity in each individual studies, which could be used to evaluate the total diagnostic accuracy. The existence of heterogeneity was assessed by the Q test and I^2 statistics. P -value of the Q test less than 0.05 or I^2 value higher than 50% indicate the random-effect model should be applied [23, 24]. In addition, Deeks' funnel plot has been used to test the publication bias, which is a vital concern for meta-analyses of diagnostic accuracy studies [25]. All analyses were accomplished with STATA 12.0 software.

Results

Literature screening process

The results of our literature screening are shown in **Figure 1**. The original search returned

MiRNAs for lymphoma detection

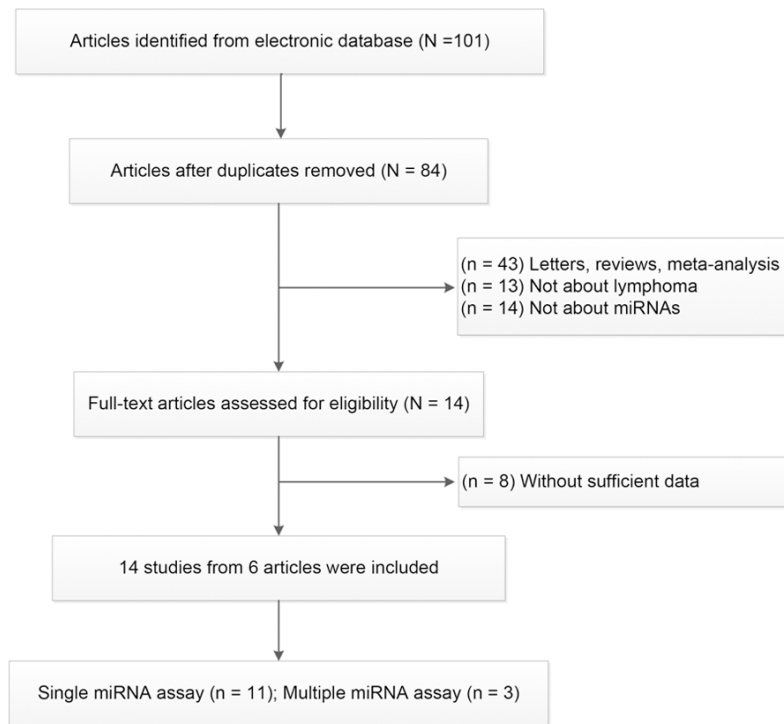


Figure 1. Flow sheet of study selection process.

a total of 101 articles from databases and other resources. Among those articles, there were 17 duplications which excluded later. After review titles and abstracts of remaining 84 articles, 70 articles were excluded (43 were letters, reviews and meta-analysis; 13 have not researched on lymphoma; 14 have not explore miRNAs). After reading the full text, 8 articles were excluded because of insufficient data. Finally, 14 studies from 6 articles meet the inclusion criteria [13, 16-18, 26, 27].

Basic characteristics of included studies

The main distinctions of six articles included in meta-analysis are displayed in **Table 1**. All included articles published from 2010 to 2014. The present analyses were based on 14 studies involving 416 lymphoma patients and 293 controls. Lymphoma patients were comprised of NHL, PCNSL and DLBCL, while controls were consisted of healthy individuals and patients with neurologic disorders (CNS inflammation, neurocardiogenic syncope, epilepsy, and tension headache). All of these studies were based on Asian and Caucasian populations. 18 types of 13 miRNAs were obtained from included studies. The quantitative real-time reverse tr-

anscription-PCR (qRT-PCR) method was applied to measure the expression of miRNAs in all available studies. The quality assessments for the studies were evaluated by their QUADAS-2 scores separately. From those scores, we have got a average score 5, which demonstrates these included studies with sufficient quality.

Diagnostic accuracy of miRNAs in lymphoma

The sensitivity and specificity data ($I^2=92.05\%$ and $I^2=87.97\%$, respectively) in lymphoma diagnosis of these 14 studies are shown in **Figure 2**, which obviously demonstrated significant heterogeneity between studies. Therefore, the random

effects model was used in this research. The pooled parameters for this study are as follows: sensitivity, 0.91 (95% CI: 0.83-0.95); specificity, 0.84 (95% CI: 0.75-0.90); PLR, 5.50 (95% CI: 3.50-8.80); NLR, 0.11 (95% CI: 0.06-0.21); and DOR, 50 (95% CI: 19-128). When we extracted one outlier, similar sensitivity, 0.88 (95% CI: 0.81-0.93); specificity, 0.85 (95% CI: 0.76-0.91); PLR, 5.80 (95% CI: 3.40-9.80); NLR, 0.14 (95% CI: 0.08-0.24); and DOR, 43 (95% CI: 16-113) were derived. These results implicated that the miRNAs assays could differentiate lymphoma patients from controls with a relatively high accuracy. Their overall SROC curve (**Figure 3A**) and outliers excluded SROC curve (**Figure 3B**) with same AUC value, 0.93 (95% CI: 0.91-0.95), which indicated a high diagnostic accuracy of miRNAs as well. Fagan's nomogram (**Figure 4**) was used for calculating post-test probabilities with PLR value of 6 and NLR value of 0.11, which predicted the increasing inerrability about a positive diagnosis by using the value of the test.

Sensitivity analysis and publication bias

The random-effects bivariate model and bivariate normality analyses confirmed the robust-

MiRNAs for lymphoma detection

Table 1. Main characteristics of 6 studies included in meta-analysis

First author	Year	Country	Ethnicity	No. of (case/control)	Mean age (case/control)	Patients	Source of control	MiRNA profiling	Specimen	QUADAS
Guo <i>et al.</i>	2010	China	Asian	79/37	41/44	Lymphoma	Healthy controls	miR-221	Plasma	5
Baraniskin <i>et al.</i>	2011	Germany	Caucasian	23/30	64/51	PCNSL	Neurologic disorders	miR-21, -19b, -92a miR-15b, -106b, -204	CSF	6
Ohyashiki <i>et al.</i>	2011	Japan	Asian	144/37	NA/NA	NHL	Healthy controls	miR-92a	Plasma	5
Baraniskin <i>et al.</i>	2012	Germany	Caucasian	39/37	66/NA	PCNSL	Neurologic disorders	miR-21, -19b, -92a	CSF	6
Fang <i>et al.</i>	2012	China	Asian	75/77	55/54	DLBCL	Healthy controls	miR-15a, -16-1, -29c	Serum	6
Mao <i>et al.</i>	2014	China	Asian	56/75	NA/NA	PCNSL	Neurologic disorders	miR-21	Serum	5

NA, not available; PCNS, L primary central nervous system lymphoma; NHL, Non-Hodgkin's lymphoma; DLBC, diffuse large B cell lymphoma; CSF, cerebrospinal fluid, CNS central nervous system.

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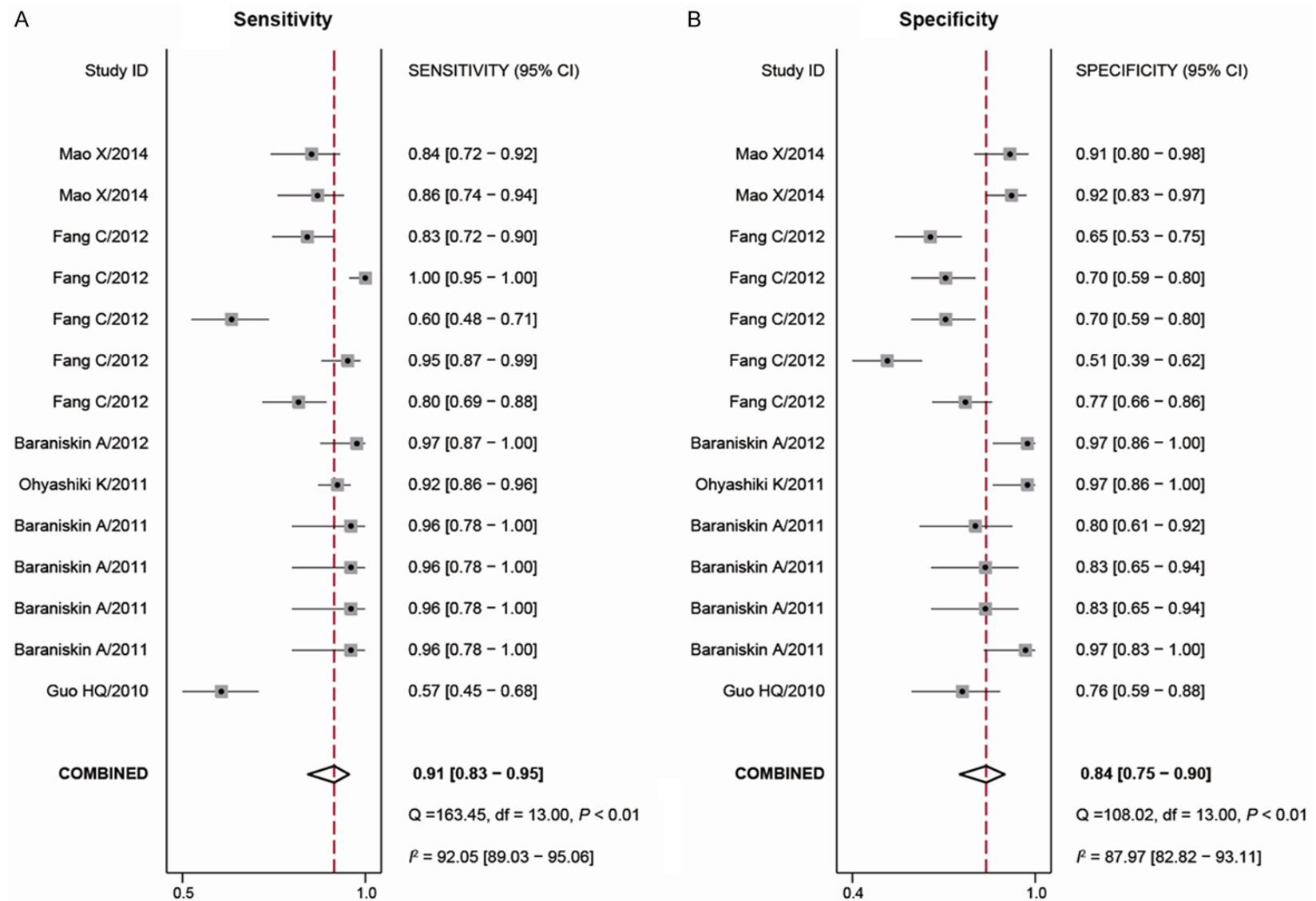


Figure 2. Forest plots of (A) sensitivities and (B) specificities of circulating miRNAs for the diagnosis of lymphoma.

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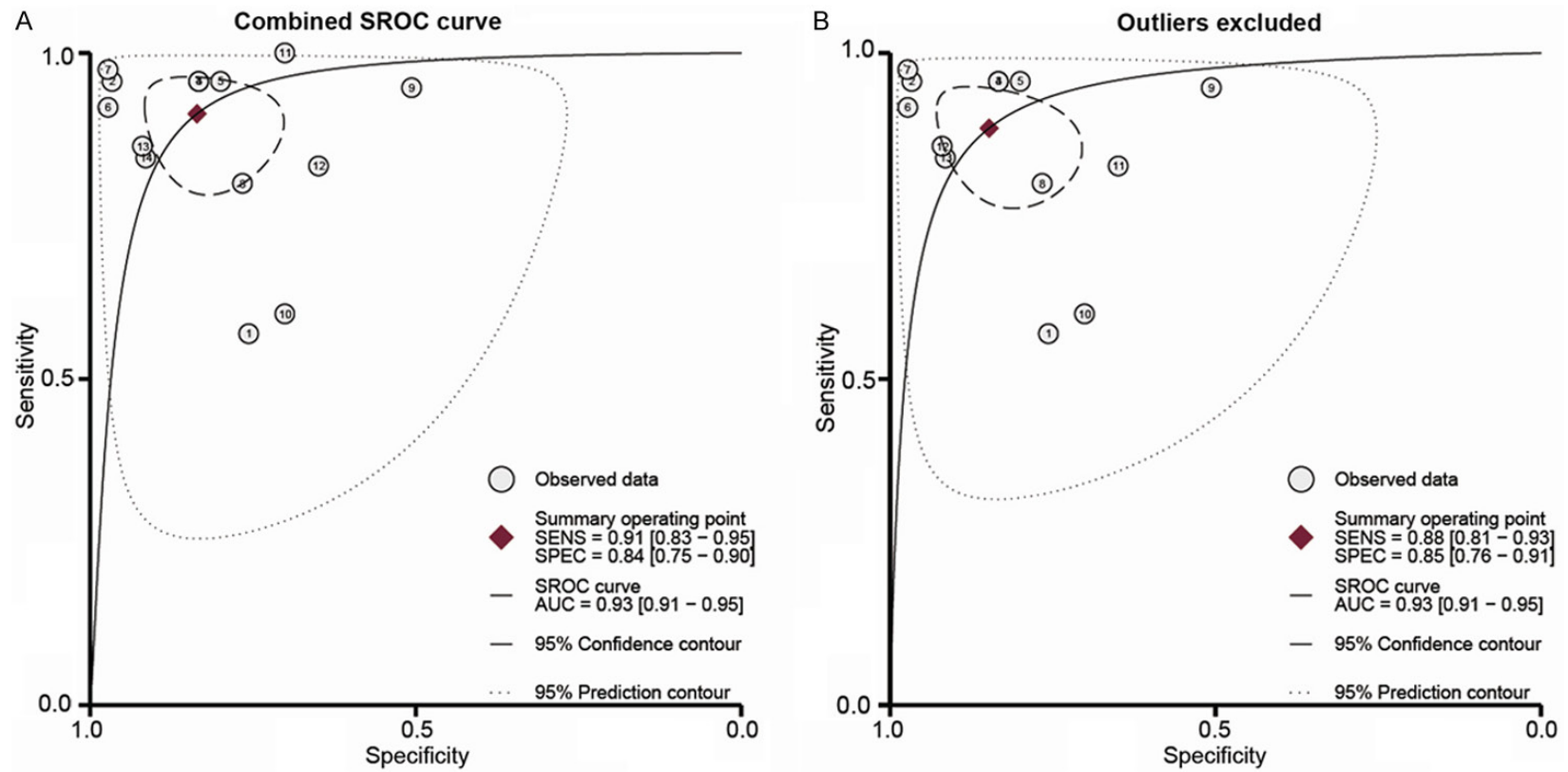


Figure 3. SROC curve with mean value of sensitivity, specificity and AUC. (A: Combined analysis; B: Outliers excluded analysis).

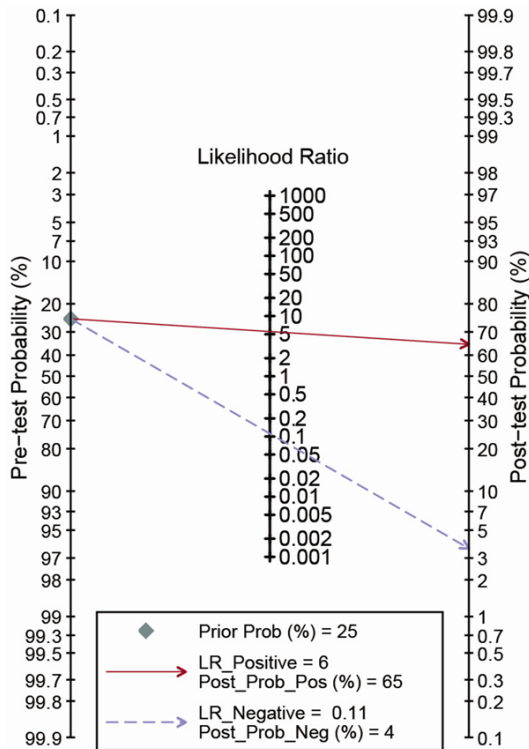


Figure 4. Fagan's nomogram to quantify the post-test probability.

ness of goodness of fit (**Figure 5A** and **5B**). Influence analysis (**Figure 5C**) and outlier detection (**Figure 5D**) illustrated there was only one outlier study [16]. The I^2 value of heterogeneity (sensitivity and specificity) and pooled assessments showed there were minimal changes after excluded the outlier study, indicating the single study has slightly influence on the overall evaluation. In order to assess the publication bias of included studies, Deeks' funnel plot asymmetry test was used. The P -value of slope was 0.12, suggesting there were symmetry and non-significant publication bias in the data (**Figure 6**).

Discussion

Although the ways of ultrasound scan, computed tomography, magnetic resonance imaging scans, biochemical examination and other histopathological diagnosis were adopted [28-31], there has no unified and effective biomarkers for lymphoma detection and risk stratification. Many reports have confirmed the abnormality of miRNAs is tightly linked to human cancers [32-35]. It was reported that some miRNAs

were closely connected with the malignant tumor disease [36]. Hence, expression analysis of miRNAs plays a crucial role in major biological processes, such as cell differentiation, homeostasis, and cancer development, which increasing the interest of cancer diagnostic and prognostic purpose. Recently, Lawrie et al. reported that various miRNAs, including miR-155, miR-210, and miR-21, were enhanced in the serum of DLBCL patients compared with healthy individuals [37]. They also suggested that the miR-21 expression might be associated with relapse free survival which could be the potential biomarkers for DLBCL. Therefore, elevation of certain miRNAs could indicate the presence of certain cancers.

Mitchell et al. found that PCNSL patients were with abundant miRNAs in their cerebrospinal fluid [38]. And the other study by Baraniskin et al. further confirmed that the expression of CSF miR-21, miR-19b, and miR-92 in PCNSL detection [17]. Guo selected miRNA-221 for their study aimed to find their potential values in diagnosis and prognosis of NK/T-cell lymphoma. Through screening samples between patients with NK/T-cell lymphoma and healthy subjects, a significantly higher plasma miR-221 level was verified in patients than healthy individuals [13]. However, it is difficult to find comparisons and contrasts between these outcomes from different studies because of the wide range of diagnostic performances of miRNAs among these studies. The various miRNAs and small sample sizes might be the primary causes for the variations and discrepancies in circulating miRNAs data among different profiling studies [37, 39-46]. In this meta-analysis, we summarized recent-findings, focusing on the potential value of circulating miRNAs as diagnostic tools in lymphoma, including NHL, PCNSL and DLBCL. To our knowledge, this is the first meta-analysis of the diagnostic value of microRNAs for lymphoma.

The pooled results based on all included studies showed sensitivity and specificity were 0.91 (95% CI: 0.83-0.95) and 0.84 (95% CI: 0.75-0.90), respectively. The value of a DOR ranges from 0 to infinity, higher values indicate a better discriminatory test performance [23]. A DOR of 1.0 indicates that a test do not discriminate patients from healthy controls. The average DOR was 50.0 in this meta-analysis which have strongly proved that miRNAs could be a useful

MiRNAs for lymphoma detection

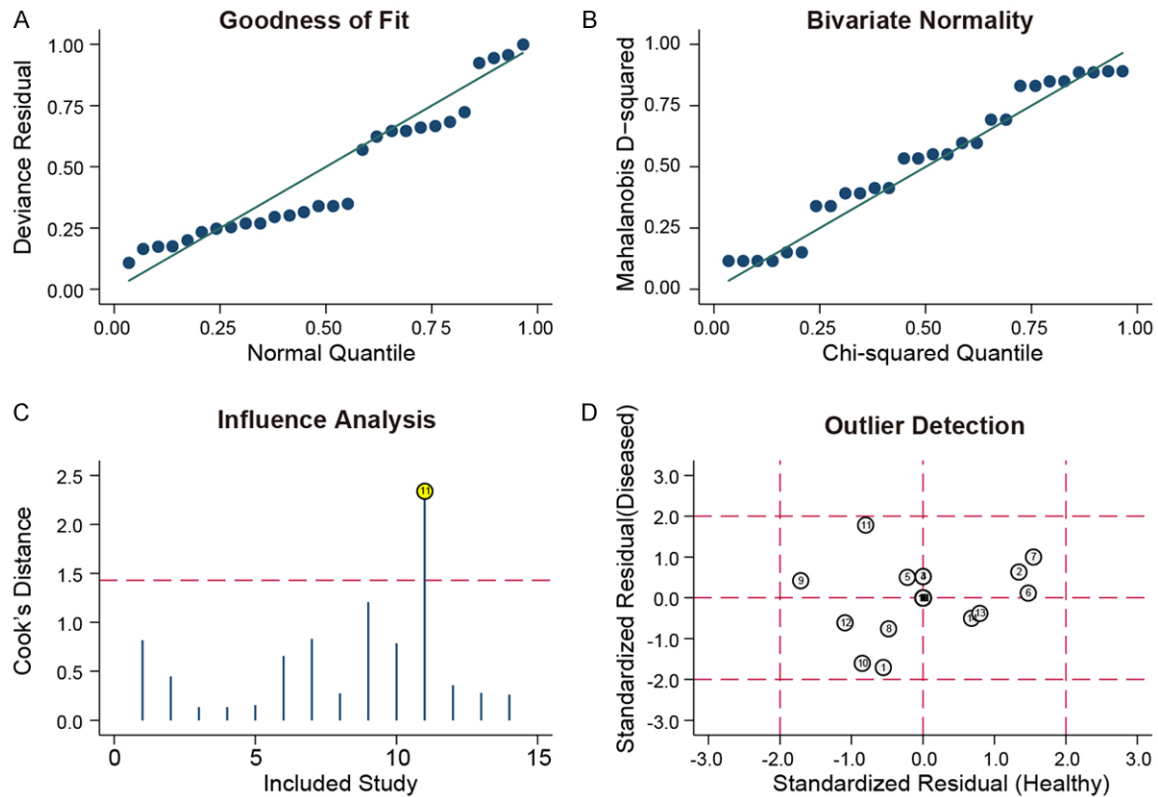


Figure 5. Graphical depiction of (A) residual-based goodness-of-fit, (B) bivariate normality, (C) influence analysis, and (D) outlier detection.

biomarker for differentiating lymphoma patients from healthy controls and those with neurologic disorders. AUC could be calculated subsequently as an alternative single indicator of test performance. The AUC, ranging from 0 to 1, higher values indicate better test performance. AUC of this test was 0.93 which reflects the overall high diagnostic accuracy of miRNA assays. Another interesting finding from **Table 1** is that multiple miRNAs assays might give significantly higher diagnostic values than single miRNA assays. Similar tendency could be observed in other human cancers, such as breast cancer, prostate cancer, gastric cancer, and lung cancer. These results imply that multiple miRNAs could offer a more accurate diagnosis.

Heterogeneity is a non-ignorable factor in meta-analysis. According to our sensitivity analysis, after removing one outlier, heterogeneity (I^2 value from 92.05 to 87.97), sensitivity (from 0.91 to 0.88) and specificity (from 0.84 to 0.85) showed tiny changes, indicating that our analysis was observed with a significant heterogeneity. Although we have not conduct meta-regres-

sion because of small sample size, potential sources of heterogeneity, including differences in country, source of controls, miRNA profiling and specimen. Results from **Table 1** suggested that there were no significant differences in the effectiveness of miRNA assays between ethnicity and source of controls. Different sample types might have different test ability of lymphoma diagnosis [36-38, 47-49]. One possible explanation for above differences is miRNAs might have varying levels of stability in different sample types and detection conditions.

For all we know, no previous meta-analysis described the overall accuracy of miRNAs in lymphoma diagnostic test. Our study is the first meta-analysis to roundly assess the diagnostic value of miRNAs for lymphoma detection, and provide the rationale for future investigations. However, there were several limitations existed in this meta-analysis when we interpret our results. Firstly, study sizes were relatively small with only six articles, thus it is necessary to strengthen our conclusion through containing further studies with large sample sizes. Secondly, all studies were conducted in Asian

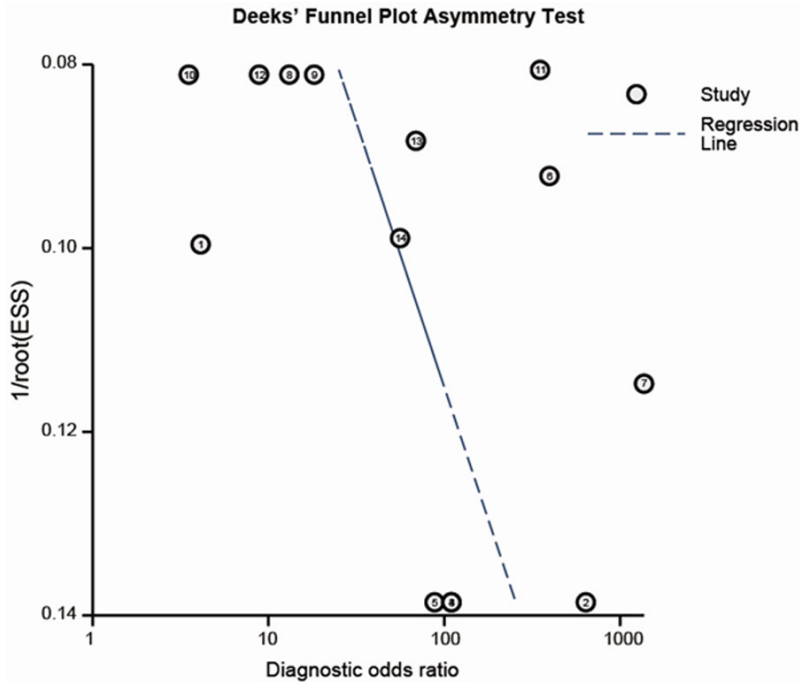


Figure 6. The linear regression of Deeks' funnel plot asymmetry test.

and Caucasian populations, so that our results could contain population selection bias. Thirdly, although best efforts have been made to gather all relevant literatures by a professional search without language restriction, we could possibly miss some researches in our screening process. Last but not least, we have not extract cut-off values due to limited sample size and various criteria, contradictory results from different studies might lead to inconsistent cut-off values.

In conclusion, the results of this meta-analysis confirmed the miRNA assays as a promising approach for screening and confirming lymphoma patients with its minimal invasive feature. Our analysis results could be served as basis for further investigation, while large prospective studies and additional improvements are still needed in order to apply miRNAs as a rapid noninvasive screening tool for lymphoma detection in routine clinical test.

Disclosure of conflict of interest

None.

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