Early Diagnostic Value of Circulating MiRNA-21 in Lung Cancer: A Meta-Analysis

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Early Diagnostic Value of Circulating MiRNA-21 in Lung Cancer: A Meta-Analysis

Chang Liu, Youping Deng*, Leilei Wang, Yong Mei, and Rui Zhang

Abstract: To evaluate the early diagnostic value of circulating miRNA-21 in diagnosis of lung cancer, databases such as Wan Fang, VIP, PubMed, and Elsevier were systematically searched from 2005 to 2013 to collect relevant references in which the diagnostic value had been evaluated. The statistics were consolidated and the qualities of the studies were classified. The data were analyzed using Meta Disc1.4 software. The diagnostic value of circulating miRNA-21 in lung cancer was assessed by pooling sensitivity, specificity, the likelihood ratio, and the Summary Receiver Operating Characteristic (SROC) curve. Publication biases of the studies involved were analyzed using Stata 11.0 software. A total of 143 papers were collected of which 8 were included, which contained 600 cases and 440 controls. A heterogeneity test proved the existence of homogeneity in this study. Upon analysis using random effects models, the weighted sensitivity was 0.68, the specificity 0.77, the positive likelihood ratio 2.84, the negative likelihood ratio 0.40, and the SROC Area Under the Curve (AUC) was 0.8133. Further analysis by subgroup showed that the 5 indicators mentioned above were 0.72, 0.84, 4.50, 0.27, and 0.8987, respectively, for the serum group and 0.63, 0.70, 1.95, 0.53, and 0.7318, respectively, for the plasma group. We conclude that circulating miRNA-21 can be regarded a valuable reference in diagnosis of lung cancer. This research showed that in lung cancer the early diagnostic value of miRNA-21 in serum was better than that in plasma.

Key words: microRNA; early diagnosis; lung cancer; meta-analysis

1 Introduction

Lung cancer is one of the malignant tumor diseases that threaten human health in modern society. The incidence and mortality rate of lung cancer rank first among various cancers across the world[1]. Once diagnosed, lung cancer often reaches the terminal stage rapidly, and thus patients lose the best treatment time. At present, the gold standard for diagnosing lung cancer definitely is still pathological diagnosis in clinics. Chest X-ray and CT scanning are often used to screen for early lung cancer patients, but the problems of low sensitivities and specificities exist[2]. Studies have shown that the false positive rate of diagnosis is close to 50% upon use of conventional methods for the detection of suspicious nodules, and unnecessary invasive examination and follow-up may grow accordingly as well[3]. miRNA is a single-stranded non-coding RNA consisting of 21-23 nt bases, whose direct complementary binding to the target gene mRNA may lead to target mRNA molecular degradation or translational inhibition, which as a result decreases the expression of target genes, and in this way miRNA participates in the process of proliferation, differentiation, apoptosis, and cell death[4-6]. Compared with the existing lung cancer test method, detection of miRNA is expected to become a method for early diagnosis. Recently, much research has been performed regarding the effect of detection of circulating miRNA-
21 on the early diagnosis of lung cancer in China and abroad. Though the results showed varying degrees of certainty, there were some limitations in the extensive use of circulating miRNA-21 detection because sample sizes of the independent research studies were too small. Therefore, to scientifically evaluate the early diagnostic value of miRNA-21 in diagnosing lung cancer, we collected pertinent literature articles to perform a meta-analysis. Based on the principle and method of epidemiology, the articles that conformed to the standard were evaluated and selected for quantitative synthesis to get a comprehensive and reliable result[7].

2 Materials and Methods

2.1 Source of material
Databases such as Wan Fang, VIP, Pub Med, and Elsevier were systematically searched from 2005 to 2013 to collect relevant references in which the diagnostic value of circulating miRNA-21 in lung cancer was evaluated. The English key words for research were lung cancer and miRNA. The Chinese key words were lung cancer, detection, and miRNA. Supplement retrieval of the references in the literature avoided the leak of some references by means of network retrieval.

2.2 Standard of inclusion and exclusion
Inclusion criteria include: (1) the diagnostic study type; (2) the diagnosis value of circulating miRNA-21 in lung cancer as a research theme; (3) patients definitely diagnosed by the gold standard test; (4) complete data extracted via the original material; and (5) language limited to Chinese and English. Exclusion criteria include: (1) literature as abstracts, lectures, commentary, and review studies; (2) a sample size of less than 40; (3) whole or part of the lung cancer patients not confirmed using the gold standard method; and (4) repeat reports, reports with small amount of information, and literature that cannot be used.

2.3 Quality evaluation of the literature
Quality classification of the literature include: (1) whether the gold standard method was set up; (2) whether the gold standard test stayed independent of the evaluation test; (3) whether the blind method was used; (4) whether data was provided for calculating sensitivity and specificity; (5) whether the right method was chosen to determine the critical value; (6) whether the diagnostic test steps were detailed; (7) whether patients with all kinds of lung cancer were included; and (8) whether internal controls were used in the process of testing. According to the above standards, we classified the qualities of the research into five grades: A, meets all quality standards; B, meets 7 standards; C, meets 6 standards; D, meets 5 standards; E, meets 4 standards.

2.4 Data extraction
The author(s), published time, data, sample size, and test method were extracted.

2.5 Statistical analysis of data
Heterogeneity test: Heterogeneity was analyzed using Meta Disc 1.4 software. If there was no significance in heterogeneity ($P > 0.05$), a fixed effects model was chosen. If it was the opposite ($P < 0.05$), a random effects model was chosen. The weighted sensitivity, specificity, positive likelihood ratio and negative likelihood ratio diagnostic advantage, and its 95% Confidence Interval (CI) were calculated using Meta Disc 1.4 software, and also using the software the SROC curve was analyzed to estimate the AUC.

3 Results

3.1 Basic characteristics of the research
A total of 143 references from January 2005 to April 2013 were retrieved initially, and a total of 9 references conformed to the inclusion criteria; one piece was ruled out because the sample size was less than 40, leaving 8 references to be included at the end of which 7 references were in English and one was in Chinese. Two pieces of data were extracted from Tang et al.[14] and one piece from the other 7 references; as a result, 9 pieces of data including a total of 1040 subjects were ultimately analyzed. Basic characteristics of the research are shown in Table 1.

3.2 Quality evaluation of the research
All research studies were based on the gold standard of pathological diagnosis; independent in the evaluation test, provided data for calculating sensitivity and specificity, and described clear experimental steps. Six studies used a correct method to determine the critical value; the remaining 2 pieces lacked detailed description. The experimental groups in 5 studies were for lung cancer patients, the other 3 for non-small cell lung cancer; internal control was used in the process of
testing in 3 articles, while the rest did not use or did not mention it; whether blind method was used is not clear in the references. The quality evaluation results for the research are shown in Table 1.

### 3.3 Basic data in the studies

The 9 pieces of data obtained from the 8 articles, types of samples, and the original data extracted are shown in Table 1.

### 3.4 Meta analysis of combined statistics

The Spearman correlation coefficient of logarithm of sensitivity and specificity was 0.133, $P = 0.732$, showing that there was no threshold effect. The Cochran $Q$ value of Diagnostic Odds Ratio (DOR) was 36.25, $P = 0.0000$, suggesting the presence of no threshold effect. Because there was significance in heterogeneity ($P < 0.05$), we chose a random effects model. For the 9 pieces of data, the weighted sensitivity was 0.68 (95% CI 0.64-0.72), specificity 0.77 (95% CI 0.72-0.80), Positive Likelihood Ratio (PLR) 2.84 (95% CI 2.03-3.96), Negative Likelihood Ratio (NLR) 0.40 (95% CI 0.29-0.56), and SROC AUC was 0.8133, as shown in Fig. 1.

### 3.5 Meta regression analysis

Meta-analysis results of the combined 9 pieces of data showed heterogeneity among the various research studies caused by the non-threshold effect. The results of exploring the sources of heterogeneity by meta-regression analysis showed for sample factors $P = 0.0029$, which means that the heterogeneity was related to the sample.

### 3.6 Subgroup analysis

The 9 pieces of data were divided into 2 groups: A, plasma group, and B, serum group. For plasma group, the weighted sensitivity was 0.63 (95% CI 0.57-0.69), specificity 0.70 (95% CI 0.63-0.76), PLR 1.95 (95% CI 1.57-2.4), NLR 0.53 (95% CI 0.40-0.7), and AUC 0.7318. For the serum group, the indices mentioned above were 0.72 (95% CI 0.67-0.77), 0.84 (95% CI 0.79-0.89), 4.50 (95% CI 3.28-6.18), 0.27 (95% CI 0.13-0.57), and AUC 0.8987. Sensitivity and specificity of the serum group were higher than those of the plasma group, and AUC was larger for the serum group. All these indicated that in lung cancer the early diagnostic value of miRNA-21 in serum was better than the value in plasma.

### 3.7 Publication bias analysis

Funnel plot analysis of publication bias (Fig. 2) of this analytical study of the early diagnostic value of circulating miRNA-21 in lung cancer showed $P = 0.94$, prompting a low likelihood of publication bias.

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**Table 1** Summary of included studies.

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Research design</th>
<th>MicroRNA assay</th>
<th>Test Control</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Specimen</th>
<th>Quadas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shen et al.</td>
<td>2011</td>
<td>Diagnostic test</td>
<td>Real-time qRT-PCR</td>
<td>58</td>
<td>29</td>
<td>46</td>
<td>12</td>
<td>19</td>
<td>1 E</td>
<td>E</td>
</tr>
<tr>
<td>Shen et al.</td>
<td>2011</td>
<td>Diagnostic test</td>
<td>qRT-PCR</td>
<td>76</td>
<td>80</td>
<td>43</td>
<td>29</td>
<td>51</td>
<td>1 D</td>
<td>D</td>
</tr>
<tr>
<td>Wei et al.</td>
<td>2011</td>
<td>Diagnostic test</td>
<td>Real-time RT-PCR</td>
<td>63</td>
<td>30</td>
<td>48</td>
<td>9</td>
<td>15</td>
<td>1 D</td>
<td>D</td>
</tr>
<tr>
<td>Zhang et al.</td>
<td>2012</td>
<td>Diagnostic test</td>
<td>Real-time RT-PCR</td>
<td>129</td>
<td>83</td>
<td>100</td>
<td>12</td>
<td>29</td>
<td>71</td>
<td>D</td>
</tr>
<tr>
<td>Wang and Zhang</td>
<td>2012</td>
<td>Diagnostic test</td>
<td>Real-time qRT-PCR</td>
<td>31</td>
<td>39</td>
<td>27</td>
<td>4</td>
<td>29</td>
<td>2 D</td>
<td>D</td>
</tr>
<tr>
<td>Li et al.</td>
<td>2012</td>
<td>Diagnostic test</td>
<td>Real-time qRT-PCR</td>
<td>82</td>
<td>50</td>
<td>39</td>
<td>6</td>
<td>43</td>
<td>44</td>
<td>D</td>
</tr>
<tr>
<td>Tang et al.</td>
<td>2013</td>
<td>Diagnostic test</td>
<td>Real-time qRT-PCR</td>
<td>62</td>
<td>60</td>
<td>30</td>
<td>13</td>
<td>22</td>
<td>47</td>
<td>E</td>
</tr>
<tr>
<td>Tang et al.</td>
<td>2013</td>
<td>Diagnostic test</td>
<td>Real-time qRT-PCR</td>
<td>34</td>
<td>32</td>
<td>18</td>
<td>9</td>
<td>16</td>
<td>23</td>
<td>E</td>
</tr>
<tr>
<td>Abd-El-Fattah et al.</td>
<td>2013</td>
<td>Diagnostic test</td>
<td>Real-time qRT-PCR</td>
<td>65</td>
<td>37</td>
<td>56</td>
<td>5</td>
<td>9</td>
<td>32</td>
<td>C</td>
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</tbody>
</table>

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**Fig. 1** SROC curve of circulating miRNA-21 for the diagnosis of lung cancer. The size of each solid circle represents the size of each study in the meta-analysis. The regression SROC curve indicates the overall diagnostic accuracy.
Fig. 2 Funnel plot. The statistically nonsignificant $p$-value of 0.94 for the slope coefficient suggests symmetry in the data and a low likelihood of publication bias.

4 Conclusions

The results of this study showed that in diagnosis of lung cancer with circulating miRNA-21, the weighted sensitivity was 0.68, suggesting that the rate of missed diagnosis was 0.32, and the weighted specificity was 0.77, prompting that the missed diagnosis rate was 0.23. The likelihood ratio is a compound index reflecting both sensitivity and specificity. Research has shown that when $PLR > 10$ and $NLR < 0.1$, the likelihood ratio has a convincing diagnostic effect, and when $PLR > 5$ and $NLR < 0.2$, it has strong diagnostic efficacy$^{[17]}$. In this study, the combined PLR was 2.84, indicating that by detection of circulating miRNA-21 in patients with lung cancer, the opportunity of positive results was 2.84 times more than that in normal persons; the combined NLR was 0.40, showing through this test that the frequency of a false negative judgment was 0.4 times more than that of a correct judgment. Furthermore the size of SROC AUC can be calculated to display the accuracy level of the diagnostic test$^{[17]}$. A certain degree of value could be certified in early diagnosis of lung cancer with circulating miRNA-21 when AUC = 0.8133. The subgroup analysis of the samples, for the early diagnostic value of miRNA-21 in serum, showed that the weighted sensitivity was 0.72, specificity 0.84, $PLR \ 4.50$, $NLR \ 0.27$, and $AUC \ 0.8987$. While for the early diagnostic value of miRNA-21 in plasma, the 5 indices mentioned above were 0.63, 0.70, 1.95, 0.53, and 0.7318, respectively. Comparison between the two groups illustrated that in lung cancer the early diagnostic value of miRNA-21 in the serum was better than the value in plasma.

Baseline similarity of the pertinent literature was good. The funnel plot indicated an imminent possibility of publication bias. And by subgroup analysis, the research showed that in lung cancer the early diagnostic value of miRNA-21 in the serum was better than that in the plasma. But the research still had the following problems: (1) the majority of the included papers did not use the blind method, leading to a measurement bias; (2) the languages of the papers were Chinese and English, which could not exclude the possibility of a language bias. More large-sample and double-blind statistical tests will be needed in the future to increase the accuracy of using miRNA-21 in early diagnosis of lung cancer. In the experiment by Shen et al.$^{[9]}$, by combining detection of miR-21, miR-126, miR-486-5p, and miR-210 in plasma for early diagnosis of Non-Small Cell Lung Cancer (NSCLC), the sensitivity was 0.86 and specificity 0.96. Chen et al.$^{[18]}$ drew the conclusion that the sensitivity and specificity of 10 serum miRNA combined detection in the early diagnosis of NSCLC reached 0.92 and 0.90, respectively. So we should focus on exploring circulating miRNAs whose expression changes are significant in lung cancer, and then through detections of multiple miRNAs or combination with other tests such as X-ray and CT, the sensitivity and specificity of early diagnosis of lung cancer will improve to a certain degree. Under these circumstances, early detection and early treatment will improve the survival rate of patients with lung cancer.

References


Youping Deng received his PhD degree in 1998 from Peking Union Medical College in 1998 and completed his postdoctoral study at Wayne State University. He is currently the Director of Systems Biology and Bioinformatics at Wuhan University of Science and Technology. His research interests include bioinformatics, biostatistics, genomics, cancer, and systems biology. He has published more than 130 papers in reputed journals and is serving as an editorial board member of 5 international journals.

Chang Liu received MBBS degree from Hubei University of Traditional Chinese Medicine in 2011 and now is pursuing her master degree in Wuhan University of Science and Technology. Her interest is epidemiology and Health Statistics and she is dedicated to the study of biostatistics, genomics, cancer, and systems biology.

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