A meta-analysis of serum p16 gene promoter methylation for diagnosis of nonsmall cell lung cancer

Yan R*, Chi L*, Zheng X†, Sun R, You J, Ye X

Departments of Occupational Disease and *Respiratory Medicine, The Second Affiliated Hospital of Shandong University of Chinese Medicine, Jinan City, Shizhong District, Shandong Province 250001, †Department of Basic Science, School of Traditional Chinese Medicine, Capital Medical University, Youanmenwai, Xitoutiao, Fengtai District, Beijing100069, China

*Yan Rongdi and Che Li contribute equally to this work

Abstract

OBJECTIVES: To evaluate the diagnostic value of serum p16 gene promoter methylation for diagnosis of nonsmall cell lung cancer (NSCLC).

MATERIALS AND METHODS: By searching the databases of PubMed and CNKI, we included all the published articles related serum p16 gene promoter methylation and nonsmall lung cancer. The true positive, false positive, false negative, and true negative data for each included publication were extracted by the reviewers. The diagnostic sensitivity, specificity, positive likelihood ratio, negative likelihood ratio, and area under the receiver operating characteristic (ROC) were pooled by MetaDiSc1.4 software. RESULTS: Finally, 13 manuscripts with 1440 subjects were involving in this diagnostic meta-analysis. The pooled sensitivity and specificity were 0.25 (95% confidence interval [CI]: 0.18–0.32) and 0.95 (95% CI: 0.93–0.97), respectively, with randomized effect model. The pooled positive likelihood ratio and negative likelihood ratio were 5.08 (95% CI: 3.00–8.62) and 0.69 (95% CI: 0.62–0.77) with fixed effect model and randomized effect model, respectively. The diagnostic ROC curve for the included 13 publications was pooled by statistical software MetaDiSc14.0 according to the Bayes theorem. The pooled area under the ROC was 0.72 with its standard error of 0.10. CONCLUSION: According to the published articles, high specificity and low sensitivity were found in this meta-analysis for the p16 gene promoter methylation in the diagnosis of NSCLC.

Key Words: Diagnosis, meta-analysis, methylation, p16 gene

Introduction

Lung cancer, the leading cause of cancer-related death, leads to 1.4 million death worldwide in the year 2008. Despite the development in aspects of diagnosis and treatment of lung cancer, the mortality is still on the raise. It was reported that only about 20% nonsmall cell lung cancer (NSCLC) patients were suitable for surgery when diagnosis. Moreover, other 80% patients with advanced disease lost the opportunity for operation, which was the main method for cure treatment procedure. Therefore, the early diagnosis is essential to the prolonged survival of this disease.

Several studies have been reported the hypermethylation of tumor suppressor gene in serum of NSCLC patients that could be a potential biomarker for diagnosis of lung cancer.[1–4] However, with small patients’ number included in each study, the statistical power was limited. Moreover, no conclusive results were reached for the diagnostic value of gene promoter methylation detection as the biomarker for lung cancer. Thus, we performed this meta-analysis according to the published articles related p16 gene promoter methylation in the diagnosis of lung cancer.

Materials and Methods

Search strategy

The open published articles were searched in PubMed and CNKI databases. The searching words were: “Nonsmall cell lung carcinoma” and “methylation” as the Medical Subject Headings and corresponding free text word searching term. The title and abstract of initial identified articles were evaluated for appropriateness to the inclusion criteria. Then, all potentially relevant articles were assessed in full-text paper, and all references of included articles were further scanned for additional analysis.

Inclusion criteria and data collection

The inclusion criteria are the patients were limited to NSCLC with pathology or cytology confirmation. The p16 gene promoter methylation array was methylation-specific polymerase chain reaction (MSP), real-time MSP, and quantitative MSP. The results were the p16 gene promoter methylation status in plasma of NSCLC patients and healthy controls. Detailed information about each article was extracted by two reviewers and then checked by the third reviewer as described in the Cochrane Handbook for systematic reviews.[6]

Meta-analysis and statistical analysis

Statistical software MetaDiSc1.4 (http://www.biomedsearch.com/nih/Meta-DiSc-software-meta-analysis/16836745.html) was used to do the statistical analysis. Statistical heterogeneity was calculated by Chi-square test.[6] If heterogeneity was found (P < 0.05 or I² > 50%), the random effect method was used to pool the data. Moreover, if no significant heterogeneity was found, the fixed effect method was used.

Results

Study characteristics

Finally, according to the inclusion criteria, 13 manuscripts with 1440 subjects were involving in this diagnostic meta-analysis. Of the included 13 studies, 7 publications come from China published in Chinese and other six papers are published in English. The general characteristic of the included 13 papers was demonstrated in Table 1.

Diagnosis sensitivity and specificity

The diagnostic sensitivity and specificity ranged from 0.13 to 0.58 and 0.72 to 1.00 for the included 13 studies. Heterogeneity test indicated significant heterogeneity among the included publications for the effect size of sensitivity and specificity. Random effect model was used to pool the combined sensitivity and specificity. The pooled sensitivity and specificity were 0.25 (95%
Table 1: The main characteristics for the included 13 publications

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Country</th>
<th>n</th>
<th>Distribution</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kersting(2)</td>
<td>2000</td>
<td>US</td>
<td>18</td>
<td>7</td>
<td>0.58</td>
<td>0.72</td>
</tr>
<tr>
<td>Bearzatto(8)</td>
<td>2002</td>
<td>Italy</td>
<td>12</td>
<td>0</td>
<td>0.40</td>
<td>1.00</td>
</tr>
<tr>
<td>Kim(1)</td>
<td>2004</td>
<td>Korea</td>
<td>14</td>
<td>8</td>
<td>0.16</td>
<td>0.94</td>
</tr>
<tr>
<td>Wu(2)</td>
<td>2002</td>
<td>China</td>
<td>4</td>
<td>0</td>
<td>0.29</td>
<td>1.00</td>
</tr>
<tr>
<td>Cai Zuxun(3)</td>
<td>2003</td>
<td>China</td>
<td>15</td>
<td>1</td>
<td>0.31</td>
<td>0.98</td>
</tr>
<tr>
<td>Fujiwara(4)</td>
<td>2005</td>
<td>US</td>
<td>14</td>
<td>3</td>
<td>0.13</td>
<td>0.96</td>
</tr>
<tr>
<td>Kong Yunming(5)</td>
<td>2007</td>
<td>China</td>
<td>19</td>
<td>0</td>
<td>0.30</td>
<td>1.00</td>
</tr>
<tr>
<td>Hsu(6)</td>
<td>2007</td>
<td></td>
<td>21</td>
<td>3</td>
<td>0.41</td>
<td>0.91</td>
</tr>
<tr>
<td>Zhang Liping(11)</td>
<td>2008</td>
<td>China</td>
<td>52</td>
<td>2</td>
<td>0.55</td>
<td>0.91</td>
</tr>
<tr>
<td>Ma Xinping(9)</td>
<td>2009</td>
<td>China</td>
<td>32</td>
<td>0</td>
<td>0.52</td>
<td>1.00</td>
</tr>
<tr>
<td>Hu Zhojun(13)</td>
<td>2009</td>
<td>China</td>
<td>22</td>
<td>1</td>
<td>0.48</td>
<td>0.95</td>
</tr>
<tr>
<td>Chen Shouhu(14)</td>
<td>2010</td>
<td>China</td>
<td>39</td>
<td>0</td>
<td>0.25</td>
<td>1.00</td>
</tr>
</tbody>
</table>

TP=True rate; FP=False positive rate; FN=False negative rate; TN=True negative rate

Figure 1: The forest plot for diagnostic sensitivity

confidence interval [CI]: 0.18–0.32) [Figure 1] and 0.95 (95% CI: 0.93–0.97) [Figure 2], respectively, with randomized effect model.

Pooled positive likelihood ratio and negative likelihood ratio

The positive likelihood ratio and negative likelihood ratio ranged from 2.07 to 40.49 and 0.50 to 0.91 for the included 13 studies. The Chi-square test showed significant heterogeneity was found in the effect size of negative likelihood ratio but not in positive likelihood ratio. The pooled positive likelihood ratio and negative likelihood ratio were 5.08 (95%CI: 3.00–8.62) [Figure 3] and 0.69 (95%CI: 0.62–0.77) [Figure 4] with fixed effect model and randomized effect model, respectively.

Diagnostic receiver operating characteristic curve

The diagnostic receiver operating characteristic (ROC) curve for the included 13 publications was pooled by statistical software MetaDiSc14.0, according to the Bayes theorem. The pooled area under the ROC was 0.72 with its standard error of 0.10 [Figure 5].

Discussion

Tumor suppressor gene promoter methylation is considered as an important mechanism for its inactivation, which occurs in the early stage of the tumorigenesis for many types of cancer.[15,16] Thus, the detection of aberrant methylation of tumor suppressor genes could be a potential method for the early diagnosis of various types of cancer, including NSCLC. The p16 gene is known as one the most important tumor suppressor genes, which plays an important role in regulating the cell cycle. This gene generates several transcript variants that regulate the G1-S transition of the cell cycle.[17] In NSCLC, this gene product has been shown to be absent in about 32–70% of the cancer cells.[18] However, mutations of the p16 gene are only found to be 0–10%[19] which indicating at least 22–60% loss expression of p16 is associated with other mechanisms, including promoter hypermethylation. Many studies and meta-analysis indicated that p16 gene promoter was hypermethylated in cancer tissue and corresponding serum as compared to healthy subjects.[20] These results indicated that p16 gene promoter methylation array maybe a useful method for lung cancer diagnosis. Moreover, several studies demonstrated the potential value with relative high diagnostic specificity. However, the subjects included in each study were relative small that limited the statistical power. Here, we performed this meta-analysis to further evaluate the serum p16 gene promoter methylation for diagnosis of NSCLC. 13 manuscripts with 1440 subjects were involving in this diagnostic meta-analysis. The pooled sensitivity and specificity were 0.25 (95% CI: 0.18–0.32) and 0.95 (95% CI: 0.93–0.97), respectively, with randomized effect model. The pooled sensitivity was very low, which could not be used as a screen for lung cancer. However, the pooled diagnostic specificity was very high, which could be used as a confirmation tool for diagnostic of lung cancer. The diagnostic ROC curve for
The included 13 publications was pooled by statistical software MetaDiSc14.0, according to the Bayes theorem. The pooled area under the ROC was 0.72 with its standard error of 0.10.

Two main limitations were existed in this meta-analysis. First, significant heterogeneity was found in this meta-analysis that reduced the statistical power; second, relative low manuscript quality was existed for the paper published in Chinese.

Inclusion, according to the published articles, high specificity and low sensitivity were found in this meta-analysis for the p16 gene promoter methylation in the diagnosis of NSCLC.

References


Source of Support: Nil. Conflict of Interest: None declared.