Diagnostic value of new combination of methylated Septin9 with LMR in adenomatous polyps and stage I to IV colorectal cancer

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Research Article

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Abstract

**Purpose** To explore the diagnostic value of tumor markers, inflammatory indicators and methylated Septin9 gene (mSEPT9) alone and combination in adenomatous polyps and stage I to IV colorectal cancer (CRC).

**Methods** Data of mSEPT9, blood routine inflammatory indicators (NLR, LMR, MPV/PC), tumor markers (CEA, CA125, CA19-9) and pathological stage from 420 patients with CRC, 61 patients with adenomatous polyps and 175 healthy people from 2018 to 2022 were retrospectively analyzed. Receiver operating characteristic (ROC) curve was plotted for the assessment of diagnostic accuracy, and statistical data were processed by SPSS 26.0 software.

**Results** The levels of NLR, CEA and CA19-9 in CRC group were significantly higher than those in healthy group, with statistical significance ($P < 0.05$). The levels of LMR and mSEPT9 in healthy group, polyps group and CRC group were decreased gradually, with statistical among all groups ($P < 0.05$). With the progressive of CRC from stage I to IV, the LMR levels and Ct value of mSEPT9 decreased gradually, and mSEPT9 in stage I/II, III and IV groups was significantly different ($P < 0.05$). The sensitivity of mSEPT9 increased gradually with the progression of stage, from 56.1% in stage I to 100% in stage IV. mSEPT9 was the most valuable single indicator in the diagnosis of CRC, and its sensitivity, specificity and AUC were 70.0%, 94.3% and 0.830, respectively. LMR was the most valuable single indicator in the diagnosis of adenomatous polyps, and its sensitivity, specificity and AUC were 78.7%, 57.1% and 0.732, respectively. MSEPT9 combined with LMR was the most valuable combination in the diagnosis of CRC and adenomatous polyps, and its sensitivity, specificity and AUC were 82.3% and 82.0%, 90.3% and 54.9%, 0.917 and 0.759, respectively.

**Conclusions** The new combination of mSEPT9 with LMR showed the best diagnostic value in the whole process of CRC occurrence and development, it should be used as the first choice for high-risk population screening, adjuvant diagnosis of CRC and disease staging.

Introduction

Colorectal cancer (CRC) is a common malignant tumor of digestive tract induced by inflammation, genetic defects, pathogenic infection and other factors. However, due to the occult early symptoms of CRC, most patients are already in the middle or late stage of diagnosis, and the survival rate is not ideal [1, 2]. Therefore, adenomatous polyps in high-risk groups and early diagnosis of CRC is very important. CEA, CA125 and CA19-9 are the most commonly used blood tumor markers in the diagnosis of CRC, however, the sensitivity of a single marker is often poor, leading to missed diagnosis. In recent years, studies have shown that the occurrence and progression of CRC is closely related to the body's inflammatory response, and some inflammatory indicators can be used for diagnosis of CRC [3]. NLR, LMR and MPV/PC are common inflammatory indicators [4]. It has been reported that these inflammatory indicators have some research values in the diagnosis of liver cancer and lung cancer [5, 6], so these
inflammatory indicators may also have some value in the diagnosis of CRC, and there are few literature reports. Meanwhile, studies have shown that mSEPT9, a molecular diagnostic marker, is closely related to the occurrence and development of some cancers, and the content of mSEPT9 in peripheral blood of CRC patients is much higher than that of healthy people [7]. However, its diagnostic value in patients with colorectal polyps and CRC at different stages is rarely reported.

In order to find the most appropriate diagnostic indicators for high-risk groups and CRC patients, we studied the application value of mSEPT9, blood routine inflammatory indicators (including NLR, LMR, MPV/PC), and tumor markers (including CEA, CA125, CA19-9) individually and in combination in the diagnosis of CRC patients at different stages and patients with adenomatous polyps, so as to achieve early detection and improve survival rate.

**Methods**

**Data Collection**

Retrospectively analyzed the data of CRC patients admitted to Xiangya Hospital Central South University in Hunan Province from 2018 to 2022. Inclusion criteria are as follows: first discovery without treatment; definite pathological diagnosis and staging; no inflammatory disease; no other neoplastic diseases; no rheumatic immune or hematologic disease; no chronic disease. Finally, 562 patients were excluded, and the data of preoperative pathological results, mSEPT9 gene, blood routine and tumor markers of 420 patients with CRC were analyzed retrospectively. In addition, 61 patients with adenomatous polyps and 175 healthy people were selected as polyps group and control group. This study was conducted under the criteria set forth in the Declaration of Helsinki and was approved by the Clinical Medical Ethics Committee of Xiangya Hospital, Central South University (Approval No. 2022020223).

**Reagents and instruments**

The Septin9 gene methylation kit was from Boercheng Beijing Technology Co., LTD.; the ABI 7500 fluorescence quantitative PCR instrument was from ThermoFisher Co., LTD.; the automatic hematology analyzer was from Beckman Coulter Co., LTD.; and the SLXP-001 automatic biochip reader was from Jiangsu Sanlian Bioengineering Co., LTD.

**Blood routine inflammatory indicators detection**

A total of 2 mL of venous blood was collected, and blood routine analysis was performed by Beckman Coulter blood and body fluid analyzer, and the inflammatory indicators of NLR, LMR and MPV/PC were calculated.

**Tumor markers detection**

A total of 3 mL of venous blood was collected, and serum was isolated by centrifugation at 3,500 rpm for 5 min, then the levels of CEA, CA125, and CA19-9 were detected by automatic biochip reader.
**Septin9 gene methylation detection**

A total of 10 venous blood collected at 1350g was centrifuged for 12 minutes to obtain 3 mL plasma, genomic DNA was then extracted from the plasma using Septin9 gene methylation kit, and the basic procedures includes DNA extraction, sulfite transformation, double-stranded DNA binding, elution and PCR amplification.

**Pathological data**

Pathological examination is the gold standard for diagnosis of CRC. According to TNM staging, patients with CRC were divided into four groups, including stage I, II, III and IV.

**Statistical Methods**

SPSS 26.0 software system was used for data processing in this study. Measurement data were expressed as mean ± standard deviation (x±s), and one-way analysis of variance was used for comparison between groups. Counting data were expressed as percentage (%), and chi-square test was used for comparison between groups. The ROC curve was used for independent and combined diagnosis to determined the AUC, cut-off value, specificity and sensitivity. $P < 0.05$ was considered to be statistically significant.

**Results**

**Comparison of indicators in different groups**

As shown in Table 1, the levels of NLR, CEA and CA19-9 in the healthy group, polyp group and CRC group were gradually increased, while the levels of LMR and Cycle threshold (Ct) value of $mSEPT9$ were gradually decreased. Compared with the healthy group, the indicators of NLR, MPV/PC, CEA and CA19-9 in CRC group were statistically significant ($P < 0.05$). LMR and $mSEPT9$ indicators were significantly different among the three groups ($P < 0.05$).
Table 1
Comparison of indicators in different groups

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Healthy group (n = 175)</th>
<th>Polyps group (n = 61)</th>
<th>Colorectal cancer group (n = 420)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR</td>
<td>1.83 ± 0.56</td>
<td>2.31 ± 2.15</td>
<td>2.63 ± 2.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.205</td>
<td>0.000</td>
</tr>
<tr>
<td>LMR</td>
<td>5.00 ± 1.43</td>
<td>3.88 ± 0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.48 ± 1.42&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>74.027</td>
<td>0.000</td>
</tr>
<tr>
<td>MPV/PC</td>
<td>0.0453 ± 0.0136</td>
<td>0.0465 ± 0.0127</td>
<td>0.0416 ± 0.0182&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.256</td>
<td>0.015</td>
</tr>
<tr>
<td>CEA</td>
<td>1.40 ± 0.96</td>
<td>1.74 ± 1.45</td>
<td>15.56 ± 61.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.227</td>
<td>0.002</td>
</tr>
<tr>
<td>CA19−9</td>
<td>7.87 ± 6.63</td>
<td>11.14 ± 12.20</td>
<td>44.19 ± 172.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.810</td>
<td>0.008</td>
</tr>
<tr>
<td>CA125</td>
<td>9.12 ± 4.66</td>
<td>8.21 ± 3.53</td>
<td>21.17 ± 137.60</td>
<td>0.940</td>
<td>0.391</td>
</tr>
<tr>
<td>mSEPT9</td>
<td>44.68 ± 1.32</td>
<td>43.23 ± 3.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.24 ± 4.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>125.143</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note: a stands for comparison with the healthy group, P < 0.05; b stands for comparison with the polyps group, P < 0.05.

Comparison of indicators in different stage of CRC

As shown in Table 2, with the progressive stage of CRC from stage I to stage IV, the levels of LMR and mSEPT9 indicators decreased gradually. Compared with stage I, II and III, CEA and CA19-9 indicators in stage IV were significantly different (P < 0.05). The diagnostic sensitivity of mSEPT9 in stage I to Stage IV was 53.1% (60/113), 65.5% (112/171), 78.9% (101/128), 100% (8/8), respectively, and MSEP9 indicator in stage I/II, III and IV three groups was significantly different (P < 0.05),
Table 2
Comparison of indicators in different stage of CRC

<table>
<thead>
<tr>
<th>Indicators</th>
<th>I(n = 113)</th>
<th>II(n = 171)</th>
<th>III(n = 128)</th>
<th>IV (n = 8)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR</td>
<td>2.91 ± 3.43</td>
<td>2.49 ± 1.15</td>
<td>2.53 ± 1.25</td>
<td>2.95 ± 1.44</td>
<td>1.125</td>
<td>0.339</td>
</tr>
<tr>
<td>LMR</td>
<td>3.61 ± 1.67</td>
<td>3.44 ± 1.42</td>
<td>3.43 ± 1.17</td>
<td>3.00 ± 0.96</td>
<td>0.715</td>
<td>0.543</td>
</tr>
<tr>
<td>MPV/PC</td>
<td>0.0456 ± 0.0207</td>
<td>0.0409 ± 0.0202</td>
<td>0.0394 ± 0.0118</td>
<td>0.0369 ± 0.0127</td>
<td>2.708</td>
<td>0.051</td>
</tr>
<tr>
<td>CEA</td>
<td>7.48 ± 18.61</td>
<td>20.97 ± 90.21</td>
<td>11.79 ± 20.12</td>
<td>8.98 ± 16.34abc</td>
<td>3.767</td>
<td>0.011</td>
</tr>
<tr>
<td>CA19-9</td>
<td>24.39 ± 81.24</td>
<td>47.53 ± 218.33</td>
<td>46.16 ± 149.61</td>
<td>219.01 ± 271.75abc</td>
<td>3.304</td>
<td>0.020</td>
</tr>
<tr>
<td>CA125</td>
<td>10.24 ± 30.21</td>
<td>30.21 ± 212.89</td>
<td>17.55 ± 38.08</td>
<td>40.15 ± 32.66</td>
<td>0.559</td>
<td>0.643</td>
</tr>
<tr>
<td>mSEPT9</td>
<td>40.19 ± 4.96</td>
<td>39.52 ± 4.85</td>
<td>38.35 ± 4.16ab</td>
<td>34.03 ± 42.96abc</td>
<td>6.674</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Note: a stands for comparison with stage I, \( P < 0.05 \); b stands for comparison with stage II, \( P < 0.05 \); c stands for comparison with stage III, \( P < 0.05 \).

Comparison of diagnostic efficacy of single and combination of different indicators in the diagnosis of CRC

As shown in Table 3 and Fig. 1, the results showed that indicators \( mSEPT9 \), LMR CEA, NLR were of better diagnostic value in the differentiation of healthy group and CRC group, with over 0.7 of AUC. The highest single diagnostic indicator was the \( mSEPT9 \) gene, and the sensitivity, specificity and AUC of \( mSEPT9 \) was 70.0%, 94.3% and 0.830, respectively. As shown in Table 4 and Fig. 2, the sensitivity, specificity and AUC of blood routine inflammatory indicators combination (including NLR, LMR and MPV/PC) in the diagnosis of CRC was 60.0%, 89.7% and 0.790, respectively. The sensitivity, specificity and AUC of tumor markers combination (including CEA, CA19-9 and CA125) was 47.70%, 96.6% and 0.750, respectively. The sensitivity, specificity and AUC of \( mSEPT9 \) with CEA combination was 74.9%, 92.9% and 0.850, respectively. The sensitivity, specificity and AUC of \( mSEPT9 \) with NLR combination was 80.9%, 88.0% and 0.887, respectively. The sensitivity, specificity and AUC of \( mSEPT9 \) with LMR combination was 82.3%, 90.3% and 0.917, respectively.
### Table 3
Comparison of diagnostic value of single indicator between healthy group and CRC group

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Cut-off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
<th>AUC 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEA</td>
<td>2.805</td>
<td>46.8</td>
<td>94.9</td>
<td>0.730</td>
<td>0.691 ~ 0.769</td>
</tr>
<tr>
<td>CA199</td>
<td>11.31</td>
<td>43.2</td>
<td>78.3</td>
<td>0.647</td>
<td>0.601 ~ 0.693</td>
</tr>
<tr>
<td>CA125</td>
<td>11.555</td>
<td>27.8</td>
<td>85.7</td>
<td>0.506</td>
<td>0.459 ~ 0.553</td>
</tr>
<tr>
<td>NLR</td>
<td>2.115</td>
<td>60.2</td>
<td>76.0</td>
<td>0.709</td>
<td>0.666 ~ 0.751</td>
</tr>
<tr>
<td>LMR</td>
<td>3.635</td>
<td>58.9</td>
<td>86.3</td>
<td>0.792</td>
<td>0.755 ~ 0.829</td>
</tr>
<tr>
<td>MPV/PC</td>
<td>0.0328</td>
<td>32.2</td>
<td>84.0</td>
<td>0.592</td>
<td>0.545 ~ 0.640</td>
</tr>
<tr>
<td>mSEPT9</td>
<td>43.95</td>
<td>70.0</td>
<td>94.3</td>
<td>0.830</td>
<td>0.798 ~ 0.862</td>
</tr>
</tbody>
</table>

### Table 4
Comparison of diagnostic value of combined indicators between healthy group and CRC group

<table>
<thead>
<tr>
<th>Indicators combination</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
<th>AUC 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor markers (CEA + CA19−9 + CA125)</td>
<td>47.7</td>
<td>96.6</td>
<td>0.750</td>
<td>0.712 ~ 0.788</td>
</tr>
<tr>
<td>Blood routine (NLR + LMR + MPV/PC)</td>
<td>60.0</td>
<td>89.7</td>
<td>0.790</td>
<td>0.760 ~ 0.834</td>
</tr>
<tr>
<td>mSEPT9 + CEA</td>
<td>74.9</td>
<td>92.0</td>
<td>0.850</td>
<td>0.820 ~ 0.881</td>
</tr>
<tr>
<td>mSEPT9 + NLR</td>
<td>80.9</td>
<td>88.0</td>
<td>0.887</td>
<td>0.861 ~ 0.913</td>
</tr>
<tr>
<td>mSEPT9 + LMR</td>
<td>82.3</td>
<td>90.3</td>
<td>0.917</td>
<td>0.895 ~ 0.939</td>
</tr>
</tbody>
</table>

### Comparison of diagnostic efficacy of single and combination of different indicators in the diagnosis of adenomatous polyps

As shown in Table 5, the highest single diagnostic indicator in the diagnosis of adenomatous polyps was the *mSEPT9* gene, and the sensitivity, specificity and AUC of *mSEPT9* was 78.7%, 57.1% and 0.732, respectively. The *mSEPT9* with LMR combination had the highest value in the diagnosis of adenomatous polyps, with sensitivity, specificity and AUC reaching 82.0%, 54.9% and 0.759, respectively.

### Table 5
Comparison of diagnostic efficacy of single and combination of different indicators in the diagnosis of adenomatous polyps

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Cut-off</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
<th>AUC 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>mSEPT9</td>
<td>44.88</td>
<td>31.1</td>
<td>94.3</td>
<td>0.626</td>
<td>0.538 ~ 0.713</td>
</tr>
<tr>
<td>LMR</td>
<td>3.365</td>
<td>78.7</td>
<td>57.1</td>
<td>0.732</td>
<td>0.665 ~ 0.799</td>
</tr>
<tr>
<td>mSEPT9 + LMR</td>
<td>/</td>
<td>82.0</td>
<td>54.9</td>
<td>0.759</td>
<td>0.692 ~ 0.826</td>
</tr>
</tbody>
</table>
Discussion

CRC is one of the most common malignant tumors in the world. Due to the change of diet and living habits, the prevalence and mortality of CRC in China have gradually increased in recent years [8]. A study conducted by Simon showed that the 5-year survival rate of patients with early-stage colorectal cancer could reach 90%, while the 5-year survival rate of patients with advanced colorectal cancer was only 14% [9]. In addition, the risk of adenomatous polyps is greatly increased, but there are no effective laboratory diagnostic indicators. Therefore, it is very important to find more sensitive indicators for high-risk population screening and early diagnosis of CRC.

At present, the commonly used diagnostic methods for CRC and adenomatous polyps patients, such as colonoscopy and tumor markers, all have certain limitations. Colonoscopy combined with pathological examination is the gold standard for the diagnosis of CRC. However, colonoscopy cannot be widely used for early screening due to the invasive operation, bowel preparation, low patient compliance and high cost [10]. Traditional serum tumor markers is widely used in early CRC screening because of simplicity and acceptability. However, the sensitivity of single tumor markers commonly used clinically in CRC diagnosis is often not ideal, so a combination of multiple tumor markers is usually used to improve the diagnostic value. In this study, there was no statistically significant difference in CA-125 level between the healthy group, polyp group and CRC group, but CEA and CA19-9 levels showed a gradual increasing trend among the three groups, and there was significant statistical difference between the healthy group and the CRC group (P < 0.05). Moreover, CEA and CA19-9 levels in stage IV were significantly higher than those in stage I, II and III. Therefore, CEA and CA19-9 indicators are of certain value in the diagnosis of advanced CRC patients. However, the sensitivities of CEA, CA19-9 and CA125 in the diagnosis of early CRC ranged from 27.8–46.8%, which was too low. Therefore, there is a very large risk of missed diagnosis, which further proves that a single tumor marker is of poor diagnostic value for CRC. In addition, the sensitivity of the combination of three tumor markers (CEA, CA19-9 and CA125) in the diagnosis of CRC was still only 47.7% in this study. Both single and combined tumor markers limited the diagnostic value of CRC. Therefore, it is necessary to find more sensitive diagnostic indicators.

In recent years, more and more studies have been conducted on the relationship between inflammatory response and tumors. Inflammatory environment may inhibit the body's anti-tumor immune response, and promote the proliferation and metastasis of tumor cells [11]. The higher the degree of malignancy of the tumor, the less the number of lymphocytes in the body, and the increase of monocytes often indicates poor prognosis of the patient, so NLR and LMR are the most direct markers of inflammation and are closely related to anti-tumor immune response [12]. Studies have found that NLR and LMR can be used as prognostic evaluation in esophageal carcinoma [13]. Wang et al. found that the level of MPV/PC was significantly reduced in patients with lung cancer [14]. It has also been reported that NLR indicator may also have certain diagnostic value in CRC screening [15].

Compared with the healthy group, in this study the CRC group showed a significant increase in NLR and a significant decrease in MPV/PC, with statistical significance. Meanwhile, the levels of LMR showed a
gradually decreasing trend among the healthy group, polyp group and CRC group, with statistical significance among the groups, indicating best discriminating value among healthy people, high-risk patients and CRC patients. During the progression and staging of CRC, there were no significant differences in the levels of NLR, LMR and MPV/PC between the four stages, but the levels of LMR showed a gradually decreasing trend with the progress of stages. The differences in the levels of three inflammatory markers between different groups confirmed the existence of an obvious inflammatory response during the progression of CRC. In terms of the diagnostic value of CRC, the specificity and sensitivity of NLR is all poor, and MPV/PC has the worst sensitivity with percent of only 32.2%; the AUC of LMR was the highest among the three indicators, reaching 0.790, but its sensitivity was only 58.9%. Moreover, the combined sensitivity of the three indicators was only 60%, which did not improve significantly. Nevertheless, the sensitivity and AUC of LMR were higher than that of single and combined indicators of tumor markers, and the detection price was much lower than that of tumor markers, reflecting better comprehensive value than tumor markers. It’s also worth noting that the results of LMR indicator are greatly affected by the inflammatory response of the body and sampling factors, and there are many interfering factors as a single diagnostic indicator. Therefore, it is better to combine with other indicators to further improve the diagnostic value.

Tumor suppressor genes are closely related to the occurrence and development of cancer, and abnormal hypermethylation of tumor suppressor genes is one of the key mechanisms leading to the inactivation of tumor suppressor genes. Currently, many studies have shown that Septing9 gene is methylated in CRC tissues, but not in normal tissues, indicating that mSEPT9 is closely related to the occurrence of colorectal cancer [16, 17]. However, it has also been reported that the sensitivity of a single mSEPT9 in CRC diagnosis needs to be improved [18].

The Ct values of mSEPT9 in the healthy group, polyp group and CRC group showed a gradually decreasing trend in this study, and the difference between three groups was statistically significant. Similar to LMR indicator, mSEPT9 also showed good distinguishing value among healthy population, high-risk patients and CRC patients. In terms of the diagnostic value of CRC, the sensitivity and specificity of mSEPT9 in CRC group were 70.0% and 94.3%, respectively, and the AUC was up to 0.830. Compared with other single indicators, it was the highest single diagnostic indicator for CRC in this study. However, it should be noted that the sensitivity of single indicator of mSEPT9 was still only 70%, which was similar to the findings from Li et al [19]. Our findings demonstrate that the sensitivity of a single mSEPT9 in the diagnosis of CRC is limited in China. Further analysis of different stage of CRC, we found that the Ct value of mSEPT9 decreased gradually with the progression of CRC stage, and there was significantly difference in stage I/II, III and IV three groups (P< 0.05). When we further analyzed the sensitivity, we found that the sensitivity of mSEPT9 in early CRC of stage I was only 53.1%, but as the stage progressed, its sensitivity gradually increased, and reached 100% in stage IV. Therefore, our findings demonstrate that mSEPT9 has good diagnostic value in CRC staging, and CRC patients with advanced stage (III and IV) are more accurately diagnosed by mSEPT9 compared with those with early stage (I and II). However, its value in the early diagnosis of CRC needs to be improved.
In this study, mSEPT9, LMR, NLR and CEA were the top four indicators in the differential diagnosis between healthy people and CRC patients. However, the diagnostic sensitivity of all indicators is not more than 70%, which indicates that the single diagnostic value of all indicators commonly used in clinical practice is limited. Therefore, we further studied the diagnostic value of the combination of these four indicators. We found that the highest diagnostic value of combination was mSEPT9 with LMR between the healthy group and CRC group, whose sensitivity, specificity and AUC reached 82.3%, 90.3% and 0.917, respectively. Compared with other single indicators or combinations, although the specificity was slightly reduced, the sensitivity and AUC were significantly improved, so its comprehensive diagnostic value was the highest. At the same time, mSEPT9 with LMR combination was also the most valuable in the diagnosis of adenomatous polyps, and its sensitivity, specificity and AUC reaching 82.0%, 54.9% and 0.759, respectively, which is lower than its diagnostic value in CRC. In addition, as the tumor progressed from stage I to stage IV, both indicators showed a gradual decreasing trend. Therefore, this combination is most valuable in identifying healthy people, polyps and CRC patients, showing the best predictive value in the whole process of CRC occurrence and development.

This study has several strengths and limitations. First, CRC patients and staging were confirmed by pathological diagnosis, which guarantee the diagnostic accuracy. Second, strict admission criteria, such as first discovery without treatment, were adopted to ensure accurate data analysis and diagnosis. The major limitation in this study is the number of patients with colon polyps and each stage of CRC, especially the number of stage IV, which may affect the statistical results. Thus, further investigations with large number are warranted.

**Conclusion**

In conclusion, a single mSEPT9 can only be used as a diagnostic indicator for patients with advanced CRC in China. The combination of mSEPT9 with LMR improves the diagnostic sensitivity especially among CRC patients in early stage, showing the best predictive value in the whole process of CRC occurrence and development. Therefore, it should be used as the first choice for high-risk population screening, adjuvant diagnosis of CRC and disease staging.

**Declarations**

**Author contributions** QS are responsible for the conception and design of the study. QYQ contributed to the acquisition, analysis, and interpretation of the data. All authors approved the final manuscript version to be published. QS is the guarantor of the article.

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**Data availability** The data are available from the corresponding author upon reasonable request.

**Conflicts of interest** The authors declare that they have no conflicts of interest.
References


**Figures**

![Figure 1](image)

**Figure 1**

ROC curve of single indicator in the diagnosis of CRC
Figure 2

ROC curve of combination indicators in the diagnosis of CRC